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PRELIMINARY NOTES ON THE DEVELOPMENT OF THE WINGS IN NORMAL AND MUTANT STRAINS OF DROSOPHILA

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Communicated April 26, 1939

Many mutations are known which affect the structure of the wings in *Drosophila*, and that organ therefore appears favorable for investigations on the developmental effects of genes.

Technique.—Pupae were fixed in boiling water, which seems as satisfactory as any more refined fixative, owing to the impermeability of the pupal chitin. The wings were dissected off and stained in Delafield's haematoxylin, and examined both as whole mounts and sectioned at 7 μ. The series of normal wings were accurately timed at 25°C.; the others were not timed, but the developmental series were constructed by comparison with the normal stages. Timing of some of the mutant types will be undertaken in the near future. The great variability in developmental timing noted by Goldschmidt was not found in this material.

The Normal Development.—In the mature larva and newly formed pupa, the wing is constituted by a fold in the epithelium of the dorsal mesothoracic disc. The epithelium in this region is thick, with elongated cells, the nuclei of which lie mainly towards the outer surface, not, however, forming a single row. The wing fold breaks through the outer surface of the disc three or four hours after pupation, and soon becomes exposed on the surface of the body. In cross-sections, the thickness from exterior (eventually dorsal) to interior (ventral) is almost as great as the width from anterior to posterior. Even at this stage, the two layers of the fold have come together, nearly obliterating the cavity. The remnants of the cavity constitute the prepupal veins, which can be made out in whole mounts and more clearly in sections, as early as 6 hours after pupation. This venation is not altogether identical with that of the adult; there is a vein along each edge of the wing, in the angles of the fold; that along the anterior margin is somewhat small and intermittent, while the posterior one is larger. One

main vein runs down the center of the wing, dividing slightly distal to the middle into two branches, which correspond to those known in the adult as *L3* and *L4*; both these extend to the tip of the wing. Posterior to them, *L5* takes a course similar to that which it follows in the adult, and joins the posterior marginal vein. No cross-veins have been made out with certainty.

The wing very soon becomes flatter (broader and thinner) by the rearrangement and multiplication of its cells; this must occur rather rapidly, since wings of the same age may be in different stages of the process, which may be complete at $6^1/_4$ hours. Except over the veins, the boundary between the upper and lower surfaces of the wing seems to disappear entirely, and no central membrane, such as that described by Hundertmark in *Tenebrio*, for instance, can be seen. After about the eighth hour, the wing begins to swell by the accumulation of fluid between the two surfaces, which are gradually forced apart. During this process the external surfaces consist of a thin cuboidal epithelium, in which lie the nuclei, while the basal parts of the cells are pulled out into thin filaments stretching across the wing from the dorsal to the central side. The process continues until the wing is a thin, tensely inflated bag. It is at its height at about 17 hours, and at this time it is not clear, from the sections, whether the basal filaments which connect the two surfaces of the wing are still persisting, or whether they have all been broken. Undoubtedly most of them have disappeared, but the experimental evidence described later is difficult to understand unless one can suppose that some have persisted.

After the stage of maximum expansion, the wing begins to contract again to a flat plate. Elongated processes appear at the bases of the cells on both surfaces, and these come together so that the two surfaces are once more joined; at this time, a central membrane is clearly visible between the upper and lower surface. As the process proceeds, the veins appear, and the exact sequence of events is therefore of great importance for an understanding of the effects of genes on venation patterns. At present, the following details seem fairly well established. The approximation of the two wing surfaces begins peripherally along the margins, and progresses inwards. Before it has affected the whole wing the hollow at the basal part of the wing begins to assume a definite form, and from it the coming together of the surfaces progresses forward; thus at one stage (about 20 hours) the veins may be present both in the tip and the base of the wing, while the middle region and the extreme base are still hollow, with a considerable space between the upper and lower surfaces; the hollow space in the middle of the wing will be called the central vesicle.

The veins which appear during this approximation of the surfaces are at first quite broad and gradually become narrower. They are, essentially, those which were present in the prepupa, *L3*, *L4* and *L5*. The prepupal

anterior and posterior marginal veins are at first absent. The posterior cross-vein can be regarded as the last vestige of the central vesicle; it is at first quite extensive and gradually becomes narrower as the vesicle disappears. The anterior cross-vein appears to be formed from an anastomosis of *L*3 and *L*4, which develops into a definite vein as these two become narrower.

The approximation of the wing surfaces takes place quite rapidly, and at about 23 hours the wing, which at 17 hours was a bloated sac, has become a fairly thin blade. During the same period, there is a considerable contraction in the area of the wing viewed from the dorsal side. In whole mounts the main part of the wing has a somewhat speckled texture, which is due to the presence of numerous small lacunae between the fibrous basal ends of the cells; only at the margins and on the veins themselves is the texture more continuous, and this is caused both by the absence of fibres running across the vein-lacunae and by a greater condensation of the superficial cells, which here form a thicker epithelium with more cylindrical cells. During the next 16 hours or so, the main changes consist in (1) a gradual expansion in area, (2) an extension of the thicker epithelium to cover the entire wing and (3) the formation of veins *L*1 and *L*2. *L*1 appears as a narrow discontinuous tube within the thickness of the anterior margin; it gradually becomes wider and continuous. *L*2 can first be seen just after the disappearance of the central vesicle. At first it looks like a protrusion from the large hollow at the base of the wing, and it appears to elongate distally until it meets the just-formed *L*1. In sections it is seen to form by a fusion of the small lacunae, with a disappearance of the basal cell processes; at the same time, or perhaps even slightly earlier, its course is marked out by the thickening of the surface cells into the higher type of epithelium mentioned above.

By about 40 hours the wing is fully formed, with all its veins. It consists of a flat plate, each surface of which is an epithelium, only one cell thick, with the nuclei lying near the surface. The basal processes of the cells are not very long and those from the two surfaces are apparently fused to form a well-marked central membrane. Each cell is beginning to form a hair. This will be called the definitive wing stage.

Shortly after this stage, the wing begins to become folded. The folding is accompanied by a great increase in area and a certain inflation of the wing, which forces the surfaces apart, stretching the basal processes. The central membrane immediately disappears. At the time of emergence, the two surfaces of the wing are fairly widely separated, but strong fibres, developed from the basal processes, run from one surface to the other. During the expansion of the wing in the young imago, these fibres persist while the spaces between them become filled with fluid. After the final

contraction and drying out of the wing, the internal structures become completely obliterated.

It is apparent from this description that the wing, during pupal development, passes through two cycles of expansion, inflation and contraction; in general outlines the process is rather similar to that described in *Tenebrio* by Hundertmark,[1] with the exception that in *Drosophila* the appearance of a central membrane is ephemeral and perhaps deceptive.

Determination.—An understanding of the mode of action of wing genes requires a knowledge not only of the changing appearances of the wing but also of the underlying processes which cause them. The first step in acquiring this knowledge is to discover the time at which the various developmental processes become determined. Only rather little information about this is yet available.

We may first ask whether the appearance of the definitive venation from the inflated stage is dependent on the proper sequence of events during the contraction. Investigation of this point has been made by puncturing the inflated wing and expressing most of the contained fluid; wings treated in this way proceeded with their development and formed perfectly normal adult wings. It is clear that the question posed above must be answered in the negative. Probably the most plausible hypothesis is to suppose that even in the fully inflated stage the upper and lower surfaces are still connected by some fibres which control the way in which the approximation of the surfaces takes place.

Defect experiments on earlier stages can be made by injuring the wing with a glass needle. If injuries are made to the imaginal disc immediately after pupation, the wing may completely fail to be everted; even so it develops a histologically normal structure, though one cannot make out when the structure is complete in the sense of containing all regions of the wing. In other specimens in which eversion has occurred, the final expansion of the wing from the folded stage may not take place, and again it is difficult to trace the complete structure. One can make out, however, that parts of the margin may be missing, and this can also be clearly seen in the few operated wings which have expanded normally. One must conclude that complete regulation to form a new wing margin does not normally occur when defects are made as early as the onset of pupation.

Gene Effects.—Some twenty-four genes have been studied in sufficient detail to be mentioned here. The study relates only to the first apparent effects of these genes on the phenotype; it must be emphasized that the fundamental changes produced by the genes may occur a considerable time before they first become visible, and can only be revealed by studies on the determination of the developmental processes. The present data are to be regarded as indications of where it is profitable to look for the determinative effects of the genes.

The gene effects investigated can be classified in five groups: (1) effects on wing size and shape, (2) breakages of veins, (3) additions of veins, (4) productions of blisters, (5) curvature of the wings.

Wing Size and Shape.—(1*a*). The wings of flies homozygous for dusky (*dy*) and miniature (*m*) are smaller than normal, but unaltered in shape. Dobzhansky[2] has shown that the individual cells of the adult wing are smaller in area than normal but equally numerous. The area of the wing is found to be abnormally small throughout development, at least from the 8-hour stage and probably earlier; the area of the cells has not yet been measured earlier than the definitive wing stage, but at that time they are already smaller than normal, both in area and in thickness. The small size of miniature and dusky wings is therefore not due, as might have been thought, to a lesser expansion of the cells during the latter part of pupal life, but is initiated at an earlier stage.

(1*b*). Broad (*br*) and lanceolate-2 (ll^2) change the shape of the wings, which in the former are broader and in the latter longer and narrower than normal; the total area is little affected. The characteristic shape of broad can be seen in the prepupal wing immediately after eversion, and persists throughout development; that of lanceolate-2 is visible in the early contraction stages (c. 23 hours) but has not been detected with certainty earlier.

(1*c*). The dumpy (*dp*) wing is smaller than normal, and has a quite abnormal shape, the whole tip of the wing being broad, forming a gentle curve concave distally. In development, the wing is smaller than normal from the time of eversion, but is normally shaped until the end of the inflated stage. The characteristic shape is assumed during the contraction and approximation of the surfaces. In the adult the cells are smaller than normal in area. The mutant Blade in *Drosophila pseudoöbscura* usually causes the wing to be narrower and considerably longer than normal, but occasionally wings of the dumpy type are found, and in some individuals the two wings belong to different types. The earliest stages of this mutant have not been examined, but the wing is quite normal in the inflated stage, and is apparently pulled out of shape in the contraction; it seems that the two types of wing represent two different ways in which the abnormal contraction may occur. In the adult the cells of the dumpy type wings are smaller than those of the Blade type, even when wings of the same individual are compared. Possibly the cell area is affected by the type and amount of contraction, but the developmental phenomena relating to cell size have not been followed.

(1*d*). Several mutants are known in which the wing appears quite normal except that portions seem to have been cut off and removed. Goldschmidt[3] has explained the phenomenon by the hypothesis that, after the wing is fully formed, a degenerative process occurs which removes the ap-

parently missing portions. His evidence is based on whole mounts of pupal wings; he claims that in fairly mild cases the wing can be seen to be quite normal at an early stage and to become progressively more abnormal, whereas if the gene produces a stronger effect the degenerative changes begin earlier and the wing is abnormal from the earliest stages. In Goldschmidt's material, the developmental stage reached at a given age seems to have been very variable and the ages which he gives do not accurately identify the stages figured. An examination of his figures, however, shows that the earliest stages he used corresponded to what I have called the inflated stage. Now at this time the wing is tensely blown out into a hollow sack, and minor irregularities in its outline may disappear owing to the internal pressure. That this actually occurs can be seen in the stock Xasta, which has a deep notch at the tip of the wing; this notch is obviously present in the prepupal wing, but becomes less obvious in the fully inflated stage, only to appear again even more strongly during the contraction. The same remarks apply to Lyra (*Ly*) which produces a wing from which parts of the anterior and posterior margins seem to be missing. It appeared possible, therefore, that the appearance of a degeneration is illusory, and is simulated by the normal contraction occurring in a wing which already lacks certain portions. A careful examination was therefore made of the wings of cut-6 (*ct-6*), one of the most strongly affected cases which Goldschmidt claims to be normal in the "earliest" (i.e., inflated) stage. It is true that in the inflated stage and earlier, the wing outline is smoothly oval. But the inflated bag is somewhat narrower than normal, and the same is true for all stages back to the just-everted wing. The evidence for degeneration therefore falls to the ground, and one can conclude that the missing parts of the wing are already missing at the time of eversion. Since we have seen that the wing margin, at least, is already determined at this time, it is possible to entertain the hypothesis that the genes of this group act by altering the position of the wing fold relative to an already determined pattern of wing veins on the surface of the imaginal bud.

The lack of convincing evidence for degeneration in the mutants just considered makes it unnecessary to assume that the altered shape of dumpy wings is due to a degeneration occurring inside the wing margin, as Goldschmidt suggested.

It should be noted that the temperature-effective period for the production of vestigial-like wings as phenocopies and for alterations of vestigial wings falls during the latter part of larval life, which is the time when the folding of the wing out of the imaginal disc is occurring. The effective period for alteration in normal wings is later, in early pupal life. The mechanisms of the two effects are probably quite different, and it may be possible to demonstrate this by investigation of the cell sizes in adult wings.

Breakages of Veins.—The following genes were studied: *Radius in-*

completus (*ri*). This was studied in *D. simulans*. In the adult *L2* is missing from near the middle of the wing almost to the marginal vein; a small trace of it may occur distally attached to the marginal vein or this may be absent. During development, *L2*, which it will be remembered is formed by the fusion of lacunae after the appearance of the other veins, simply fails to extend, the lacunae becoming gradually filled up by the condensation of the wing without ever fusing into a continuous channel. The distal piece, if present, is probably formed centripetally, starting from the marginal vein and extending inwards.

Tilt (*tt*) causes a gap in *L3*. This vein is normal in the prepupal stage. At the very earliest appearance of the definitive veins, the vein appears to extend through the gap, but it disappears there almost immediately, and the region acquires the mottled texture of the loosely porous submarginal cell lying anterior to it. At a slightly later stage, the looser texture of the submarginal cell has the appearance of spilling through the gap in *L3* into the more condensed region between *L3* and *L4*. It is possible that this gene has some similarities with balloon, which also seems to slow up the condensation of the submarginal cell: Thus a tilt mutation in *D. affinis* often causes the appearance of extra veins in the submarginal cell.

Veinlet (*ve*) causes a lack of the distal ends of *L3*, *L4* and *L5*. The prepupal venation is normal, and *L3* and *L4* extend to the tip of the wing in the earliest contraction stages (*L5* has not been traced at this time). The ends of *L3* and *L4* disappear fairly soon, probably by the formation of cell processes connecting the two surfaces of the wing. At about a 30-hour stage in normal wings, the surface epithelia are thicker, with more closely packed cells, immediately above the veins than in the intermediate regions. In *ve* the strands of thickened epithelium continue beyond the ends of the lumina in *L3* and *L4*.

It is possible that the parts which fail in *ve* are those normally formed centripetally, while the intact parts are those which form from the central vesicle, but this is not yet certain.

In *cubitus interruptus* (*ci*) *L4* is broken distal to the posterior cross-vein, and *L5* proximal to it; in both cases short fragments of vein may be present. Here the effect can already be seen in the prepupal venation. In the 7- or 8-hour stage only very vague traces of *L4* can be found, and somewhat more definite traces of *L5*; this was confirmed in sections. The pupal venation has its characteristic pattern from its earliest appearance during the contraction. In another allelomorph, ci^{w}, the abnormality (total lack of *L4* distal to the cross-vein) can also be seen from the earliest contraction stages. The prepupal condition has not been investigated.

Crossveinless (*cv*) causes the absence of both anterior and posterior cross-veins. In the *cv ct-6* compound, something like the posterior cross-vein can be seen in early contraction stages, when it is still more or less part of the

central vesicle. It soon disappears. The anterior cross-vein is at first no more than an anastomosis of *L3* and *L4*. In *cv* it disappears as these veins become narrower in about the 28–36 hour period.

The Formation of Extra Veins.—There appear to be two main mechanisms by which extra veins are formed: (1) a precocious and exaggerated condensation of the surface epithelium, to give a thicker, more closely packed layer, in which supernumerary luminae appear and over which extra chitin is secreted; (2) a failure of condensation of the basal regions of the cells, which retain a porous texture, with many small luminae, right up to the stage at which the wing begins to fold, giving eventually thin chitinization with weak, straggly veins. The first process, exaggerated growth of the epithelium, occurs characteristically in the second posterior cell (i.e., posterior to *L4*, distal to the posterior cross-vein) and along the margin particularly at the tips of *L2*, *L3* and *L4*. The strongest example I have seen is in net (*net*), where the thickening of the anterior margin and the second posterior cell is so great as to give the cross-section the appearance of an elongated dumbbell. The condensed regions seem to exert a tension on the rest of the wing, since the comparatively uncondensed cell between *L3* and *L4* may be stretched in the middle region, so that the tips of *L3* and *L4* are bent towards one another. The formation of extra veins within the thickened regions reminds one strongly of the formation of *L1* along the anterior margin in normal development, since this region is normally somewhat thickened. Probably the position in which the lumen occurs is dependent on the thickness of the overlying tissue, since in the exaggerated thickenings along the margin and at the ends of *L2*, *L3* and *L4* it occurs some distance in from the edge, giving "deltas," such as those found in the mutant of that name, which acts very similarly (Dl^6 was examined). In plexus (*px*) and blistered[2] (bs^2) conditions are very similar to those in net, though less extreme, and there is some indication of a lack of condensation in the submarginal cell, which is more marked still in Dl^6, in which the adult shows a markedly thickened *L2*. This lack of condensation has been most strongly seen in balloon (*ba*). It gives rise to a weak chitinization, though in other stocks a blister may be formed here, presumably by a failure of the final unfolding and drying out of the wing. Balloon also shows some degree of thickening of the second posterior cell, and extra veins appear here; by comparison with net and plexus, the thickening hardly seems considerable enough to account for these, and some other mechanism may be involved.

In all the above, the prepupal venation is normal.

Formation of Blisters, etc.—In these cases the adult wings show more or less extensive blisters, which may remain filled with fluid or may eventually dry out; sometimes the whole wing is affected, in which case its expansion from the folded condition which it has in the late pupa may completely

fail. Some of the genes concerned seem to have no effect until the actual time of unfolding; examples are Wrinkled (*W*) and vesiculated (*vs*). Probably they act by causing the breakage of some of the fibres which normally hold the two surfaces of the wing together during the unfolding. The gene bloated (*blo*) produces a somewhat similar abnormality, but here the effect can clearly be seen in the definitive wing, though the prepupal wing is normal. The contraction from the inflated stage is very incompletely performed, and at the time the wing starts to fold it is still quite loose-textured and porous, and contains many extra pieces of vein, while the normal veins are wide and irregularly formed. There are also cysts derived from the surface epithelia, which form small hollow balls within the thickness of the wing; they eventually differentiate into spherical nodules of typical wing membrane, with the hairs pointing inwards. At the time of dissection it was noted that the *blo* pupae seemed to be particularly full of body fluid; it may be that all the abnormalities of the wings can be explained by a greater internal pressure, which prevents the normal contraction and may even cause the rupture of the wing surface, thus setting free the patches of tissue which form the cysts.

The mutation blot (*blt*) causes a faulty unfolding of the wing, but this is only a secondary effect. It actually produces a partial twinning, portions of a second wing being formed in the region proximal and posterior to *L*5 (the third posterior cell). The twin is a mirror image of the normal wing, mirrored in the plane of *L*5, but only rarely is it complete enough to include any of the stiff bristles characteristic of the anterior margin. Its venation is irregular, and not yet fully made out. The alula does not appear to be involved. The effect is highly variable, with a low penetrance.

Wing Curvature.—Studies have been made of the definitive stages of Curly (*Cy*), in which the wing is usually bent upwards, and of curved (*c*) in which it is usually bent downwards. In both cases there is no trace of curvature in the definitive stage. Probably the genes affect only the drying out of the young imaginal wing, though it is possible that studies on cell size will reveal earlier effects.

[1] Hundertmark, A., "Die Entwicklung der Flügel des Mehlkäfers Tenebrio Mollitor," *Zeits. Morph. Oekel. Tiere.*, **30**, 506 (1936).

[2] Dobzhansky, Th., "The Influence of the Quantity and Quality of Chromosomal Material on the Size of the Cells in Drosophila melanogaster," *Arch. Entwmech.* **115**, 363 (1929).

[3] Goldschmidt, R., "Gen und Aussencharakter. III," *Biol. Zbl.*, **55**, 535–554 (1935); "Gene and Character. IV," *Univ. Cal. Pub. Zool.* **41**, 277–282 (1937).

HIGH MUTATION FREQUENCY INDUCED BY HYBRIDIZATION

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Communicated June 13, 1939

The increase in variability that is a familiar result of the rearing of second generations from crosses between different races is certainly due chiefly to segregation and recombination of genes in which the parental races differed. There is, however, a persistent feeling that perhaps interracial crossing also induces the production of new mutations. The first attempt to test this view experimentally with Drosophila led to negative results (Duncan 1915). Belgovsky (1937) reported an increased frequency of somatic mutations in the hybrids between *D. melanogaster* and *D. simulans*, in x-ray experiments. The differences reported were not very striking, and in any case have little bearing on questions concerning spontaneous frequencies.

Casual observations have led me to suspect an increased mutation frequency in the hybrids between the two semi-cross-sterile races (*A* and *B*) of *Drosophila pseudoöbscura*. The present note describes experiments planned to measure the rate quantitatively in such hybrids.

Two different interracial crosses were carried out, using appropriate "marker" genes. Females of the scutellar scarlet (race *B*) stock were mated to males of the beaded (race *A*) stock. The F_1 females were mated to yellow compressed short (race *A*) males, and the daughters (first backcross—1 *BC*) of this mating were tested individually by yellow compressed short males, to give the 2 *BC* generation. In the second experiment, yellow compressed short (*A*) females were mated to pointed (*B*) males, and the F_1 females were mated to scutellar scarlet (*B*) males to give the tested 1 *BC* females. In both cases females of the 2 *BC* generation were also tested for lethals, by mating to males of the strain to which their respective fathers belonged. The table shows the sex-linked lethals recovered (referred to the generation to which the first detected heterozygous female belonged), and the number of "visible" mutant types observed in the males (referred to the generation to which the males themselves belonged—in most cases presumably one generation later than that in which the actual mutation occurred).

The totals (denominators) shown in the table represent, for lethals, twice the number of tested females. This is because 12 of the 31 recovered lethals were in paternal *X*-chromosomes, which had evidently undergone mutation soon after fertilization had introduced them to the hybrid zygote. For the visibles the total given is that of the males counted in the generation concerned. Each separate mutation is counted once, though several of them

appeared in several offspring of single mothers. These relations show that few, if any, of the mutations occurred at meiosis. They are, therefore, scarcely to be referred to crossing-over or recombination.

The "visible" types recovered were mostly previously known types. Thus, forked (or singed—probably both are present, but the tests are not yet completed) was found five times, beaded three times, scutellar twice and dusky, bobbed, ascute, slender and short each appeared once. In the

TABLE 1

DETECTED MUTATIONS

GENERATION	BACKCROSSES TO RACE *A*		BACKCROSSES TO RACE *B*		TOTAL (PERCENTAGES)	
	LETHALS	VISIBLES	LETHALS	VISIBLES	LETHALS	VISIBLES
1 *BC*	0/38	1*/124	9/66	0/243	8.7	0
2 *BC*	6/106	0/911	16/152	10/1046	8.5	0.56
3 *BC*	...	5/2135	....	4/3796	...	0.15

* This was a somatic mutation—there was a large patch of forked (or singed) bristles on the thorax. The mutation is not included in the totals, since it is not comparable to the other "visibles," which presumably occurred in the mothers of the males recorded.

4 *BC* generation (not shown in the table because the pedigrees were varied in order to carry out tests of suspected mutant types) there were found white, bobbed, miniature and another forked (or singed).

The lethal frequencies given are to be taken as minimum values. As has been shown by Lancefield and others (see Sturtevant 1937a), the sex-ratio from *A*–*B* hybrids is variable. For this reason all sex-linked lethals were tested, and none were entered in the table unless they resulted not only in a 2:1 sex-ratio but also in typical lethal ratios for the sex-linked genes heterozygous in the culture. This requirement was met in at least two successive generations. From several probable lethals no satisfactory test was obtained in the following generation, and these have not been recorded, though the chromosomes concerned do appear in the totals. Incidentally, this test also showed that the loci of the lethals were scattered throughout the *X*-chromosome.

Owing to the complications just mentioned, and to the small number of cultures reared, it is not possible to judge whether there was an increased mutation frequency in the F_1 generation. The point is of theoretical interest, in connection with the hypothesis outlined below, but will have to be studied in experiments especially designed for the purpose.

In addition to the mutations shown in the table there were three dominants in the females of the 3 *BC* generation (Smoky, an extreme minute-bristle, and a sex-linked wing character). In the regular series no autosomal recessives could have been detected, but in testing one of the early sex-linked recessives, hybrids were mated together, and among their offspring was found the well-known second chromosome recessive, bithorax.

These experiments were carried out on a small scale, and no specific control is available. Nevertheless, the mutation frequencies—about 9 per cent of lethals and one-half of 1 per cent sex-linked visibles—are so much higher than any other recorded spontaneous rates that their significance cannot be doubted. It may be added that I have detected only one sex-linked lethal in pseudoöbscura in experiments not concerned with *A–B* hybrids.

The theoretical implications of the results just described may be deduced from the effects that natural selection should have on mutation rate. I have argued (Sturtevant 1937b) that natural selection must operate to keep the general mutation rate of a species at a minimum—a conclusion also reached by Shapiro (1938). I have suggested that different populations may achieve this result by different genetic means, and that crossing such different races should, through recombination, lead to an increase in mutation frequency. The results here recorded constitute a verification of this prediction. The increase here is unexpectedly great, but it may be pointed out that the cross made is about as extreme a one as it is possible to study in later generations.

These results indicate that a species may escape the impasse toward which the automatic decrease in mutation frequency is constantly pushing it, by the occasional interbreeding of semi-isolated populations within the species.

The general method of crossing may also be of use in the study of the nature of the mutation process, since there is no indication of an increase in major chromosome aberrations such as accompany x-ray treatment. Further study will be required to answer the question whether small rearrangements (deficiencies, small inversions) occur more frequently.

Summary.—The offspring of the backcrosses from hybrids between the two races (*A* and *B*) of *Drosophila pseudoöbscura* show a large increase in mutation frequency.

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MICROGEOGRAPHIC VARIATION IN DROSOPHILA PSEUDOOBSCURA

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Studies of recent years have revealed a prodigious amount of variability in the gene arrangement in the chromosomes of several species of Drosophila. In natural populations of *D. pseudoobscura* the third chromosome is more variable than the rest; eighteen structural types, related to each other mostly as overlapping inversions, have been found in this chromosome alone.[1] Each type of the chromosome structure is restricted geographically to a part of the distribution area of the species, although in most localities several types coexist in the same population. Differences between populations are either quantitative, i.e., involving only the relative frequencies of the different types, or qualitative, when certain types occur in some localities but not in others. If population samples from a chain of localities sufficiently remote from each other are studied, the frequencies of the gene arrangements are commonly found to increase, or to decrease more or less regularly in a given geographical direction. Thus, the "Santa Cruz" arrangement is unknown in Washington and Oregon, is rare in the San Francisco Bay region, more common in southern California, especially on the islands off the coast, and very common in Mexico and in Guatemala.[1, 2] Such geographical gradients are familiar to students of geographical variation both in animals and in plants.

Differences between populations inhabiting neighboring localities seem to be of a somewhat different kind. Population samples were taken on eleven mountain ranges in the Death Valley region of California and Nevada. Only three ranges yielded samples the differences between which were not significant statistically; the others were all different. No geographical regularity is, however, observed in this variation, since adjacent ranges are no more likely to be similar than the remote ones, and vice versa. The elementary breeding unit in *D. pseudoobscura* is therefore not a population inhabiting a large territory; it is no greater than the population of a single mountain range.[3] Next, population samples were taken in six canyons of one of the mountain ranges in the same region; these samples proved to be about as different from each other as those from separate mountain ranges. The breeding unit is, consequently, no greater than the population of a single canyon,[4] and it may be much smaller than that. Comparison of populations coming from localities even more closely adjacent than the above ones is clearly in order.

A small, but very carefully labelled, collection of strains of *D. pseudo-*

obscura from Texas has been received from Professor J. T. Patterson, whose generosity the writer takes pleasure to acknowledge. Each strain represented the offspring of a single fertilized female caught outdoors; with the exception of several cultures in which the wild progenitor has remained alive, all the rest contained the first generation offspring of the original flies. Two or three females and males were taken from every culture and inbred; the chromosomes were examined in the salivary glands of from ten to twelve larvae per culture. Since a fly has two chromosomes of each kind (except, of course, the X and Y-chromosomes), the offspring of a pair of wild flies carries combinations of the four chromosomes present in the parents. In wild flies these chromosomes may be either alike or different in the gene arrangement. Examination of a sufficient number of F_1 larvae permits, therefore, to determine the gene arrangement in four wild chromosomes. The same result may be accomplished also by examining F_2 larvae, although in some instances one, or even two, of the four chromosomes may be already missing. Since our F_2 cultures have descended from at least four F_1 flies, such instances must be very rare, however. Nevertheless, the difficulty just stated does introduce some uncertainty in the data.

The gene arrangement in the second, fourth and in the left limb of the X-chromosome was found to be constant in all strains. In the right limb of the X, the triple inversion characteristic of the "sex-ratio" condition was present in some cultures.[5] Six different gene arrangements were found in the third chromosome, namely, Pike's Peak, Arrowhead, Tree Line, Olympic, Standard and Estes Park; their descriptions have been published by Dobzhansky and Sturtevant,[1] and need not be repeated here. The number of the wild third chromosomes having each of the above gene arrangements found in the different samples is as follows:

	PIKE'S PEAK	ARROW-HEAD	TREE LINE	OLYMPIC	STANDARD	ESTES PARK	CHROMO-SOMES STUDIED
Aldrich Place, near Austin	33	1	..	..	..	2	36
Carrizo Springs	6	4	1	4	..	1	16
Catarina	19	1	5	3	..	..	28
Herrington Orchard, near Dilley	37	12	14	..	1	..	64
Redus Orchard, near Dilley	15	2	5	2	..	..	24
Forrest Orchard, near Dilley	19	2	..	3	..	..	24
Three Rivers	19	1	5	3	..	..	28

The figures suggest that at least some of the samples compared are unlike in genetic composition. The question is now whether the observed dissimilarities are real, or are due to sampling errors. The validity of the suspected heterogeneity of the samples may be tested by calculating the χ^2 values for the common gene arrangements (Pike's Peak, Arrowhead, Tree Line and Olympic), using the Brandt and Snedecor method.[6] The χ^2 values are found to lie between 13.6525 (for Arrowhead) and 20.5730 (for

Pike's Peak); for six degrees of freedom, the probability of such or greater heterogeneity being due to sampling is less than 5% (for Arrowhead).

It must be noted, however, that the seven localities listed above fall into two geographical groups; Aldrich is at least 150 miles from the rest, while all others are relatively close. One may, then, disregard the Aldrich material, and test the heterogeneity in the remaining six samples. The χ^2 values turn out to vary from 7.7765 (for Tree Line) to 11.5080 (for Olympic), which, for five degrees of freedom, have a probability between 5 and 20% of occurring by chance. Finally, the three localities in the vicinity of the town of Dilley may be compared to each other. These three collecting grounds are indeed very close to each other: according to the information received from Professor J. T. Patterson, Herrington orchard lies $1^1/_2$ miles west of Redus orchard, while Forrest orchard is only $^1/_2$ of a mile north of Redus. Yet, no Olympic arrangement is detected among 64 wild third chromosomes studied from Herrington, and no Tree Line arrangement is found among 24 chromosomes from Forrest orchard. If the three localities are compared, the χ^2 values are 2.4313 for Arrowhead, 3.4403 for Pike's Peak, 5.1229 for Olympic and 6.2542 for Tree Line. For two degrees of freedom, an χ^2 value of about 5.1 has a chance of accidental occurrence between 5 and 10%, and a value 6.25 may occur due to sampling in from 2 to 5% of the trials.

Although the material at hand is clearly inadequate for an exact determination of the relative frequencies of the different gene arrangements in any one of the populations, and although the necessity of using F_2 instead of F_1 larvae introduces an additional uncertainty, still the data obtained strongly suggest a significant heterogeneity among the samples examined. The lack of similarity between the three samples from Dilley is, of course, most remarkable in view of the proximity of these collecting localities. The observations quoted above on the genetic differentiation of the local colonies of *D. pseudoöbscura* in the Death Valley region of California make the data reported in the present communication less surprising than they might have been otherwise. It may be pointed out, however, that the exceedingly rugged topography characteristic of the mountain ranges in Death Valley may offer a greater facility for the formation of isolated colonies of the fly than is likely to be the case on the relatively more uniform terrain of southern Texas. On the other hand, Professor J. T. Patterson informs me that *D. pseudoöbscura* is on the whole rare in Texas, the numbers of the trapped individuals of this species being much less than those of others; on the contrary, *D. pseudoöbscura* in California mountains is by far the commonest species. A lower population density per unit area may, under certain conditions, favor a genetic differentiation of local colonies.

Summary.—Data are presented that suggest the existence of genetic differences between colonies of *D. pseudoöbscura* inhabiting close localities.

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A REMARKABLE GROUP OF POSITION-EFFECTS

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The term position-effect is used here in the generally accepted sense though it would be advisable to replace it by a more neutral term, like "pattern-effect" which does not imply the explanation in terms of position of genes, an interpretation which does not work in the majority of cases. The effects to be described are nearly related to Dubinin's case of change of dominance of *cubitus interruptus* in the presence of a *IV-Y* translocation in *Drosophila melanogaster*.

A.—Years ago the senior author found in connection with very different work the following case: The dominant mutant Beaded (*Bd*) has been known, since Muller's well-known work, to be enhanced in its action by the presence of an enhancer somehow combined with an inversion in the homologous chromosome. When *Bd* was separated in our work from all the other chromosomes of the *Bd*-line by replacing these with marked chromosomes, the "dominant" effect of *Bd* consisted only in the production of a small per cent of flies with slightly nicked wings (actually a smaller effect than the one produced by some standard "recessive" loci like *px* or *bs*). The addition of the inversion (now called *In*(3*R*)*C*) again restored the high grade of specific scalloping called *Bd* and the percentage incidence up to 100%. It was then found that the action of this inversion could be replaced by some of the Payne-Inversions in the same chromosome, which were available at that time, thus showing that the presence of an inversion and not an enhancing gene was responsible for this effect. One of the junior authors (Gardner) has now reinvestigated this case. He finds that all inversions in the third chromosome which were tested, had the same enhancing effect, among them an inversion in the other end of the third

chromosome and the very small Dichaete-inversion, neither of which includes the *Bd*-locus. Very characteristic was the following fact observed in this connection. Oregon wild stock was generally used as a control. When, however, Florida wild stock was used; it frequently enhanced the *Bd*-effect like the inversions. A cytological study by one of the junior authors (Kodani) revealed the presence of a large inversion in the third chromosome of the Florida stock, resembling the inversion *In*(3*R*)*P*. These enhancing effects (which might also be described as increase in dominance, since *Bd* is homozygous lethal) which can be increased to the production of a wing approaching the vestigial-strap type in appearance are, then, so-called position-effects, though the variety of locations of the breaking points excludes an explanation of the effect in terms of position of genes.

B.—In the analysis of a chromosome-rearrangement at the right end of the second chromosome the so-called recessive gene blistered (*bs*) was frequently used by the senior author. This homozygous stock (as received from Pasadena) possessed as its only visible character a small extra vein, sometimes attached antler-like to the fifth vein (and this only in a certain percentage of individuals). The heterozygotes are similar except for the fact that a smaller percentage show the extra vein. In these crosses strange types of individuals appeared in a way which did not agree with other results. Finally the *bs*-stock was inspected and genetical tests showed that many of the flies contained something in a heterozygous condition which was responsible for the odd results. An examination of the salivary gland chromosomes by one of the junior authors (Kodani) revealed the presence of a reciprocal translocation between the free end of the first chromosome and the right arm of the third chromosome. This translocation was shown to have an enhancing effect on *bs*, though without any visible effect when alone. A series of compounds involving the *bs*-locus has been made up, including: standard *bs;* a new condition at the *bs*-locus which might be termed here bs^a, acting like a somewhat higher allele of *bs;* the deficiencies Px^1, Px^2 and blond-deficiency, all of which involve, among other loci, the *bs*-locus. Without going into detailed description it should be mentioned that a series could be made up of multiple allelic nature beginning with a little extra vein, which is increased to a more and more broad antler-like structure; then follows an increasing series of extra venation in other parts of the wing, followed by more and more extreme blistering. Every single member of this series of compounds is enhanced by the translocation to a much higher grade. Most characteristic is the highest grade produced by the compound *bs*/*DeF* plus translocation. Here the wings are short balloons filled with liquid and carried at right angles to the body. If bred they produce a majority of their own type (the deficiencies are homozygous lethal) and a varying small number of a new

type with a broad sinus extending from the fifth vein to the edge of the wing and a blister on one of the wings. These are completely sterile in both sexes and probably represent the homozygous translocation plus the compound. (The hyperploid or deficient type has not been excluded yet.) Details will be given in a future paper.

C.—In some earlier papers the senior author described the dominance-enhancing action of some additive modifying loci upon the vestigial /+ heterozygote. Further, certain loci were found which enhanced or decreased the action of these so-called dominigenes. In following up this work one of the junior authors (Gardner) found that this dominance-increasing action is considerably enhanced in the presence of a number of deficiencies, inversions and translocations, none of which have anything to do with the *vg*-locus. The interesting details will be published in a special paper. Only one characteristic feature may be mentioned here. It was found that the dominance enhancers as well as the still more enhancing chromatin-rearrangements all increased the time of development, thus suggesting that the action in common to all is a change of rates of certain developmental processes. This agrees well with the senior author's work on temperature action in producing the simile (phenocopy) of a *vg*-heterozygote plus dominigenes and other earlier work in the same line, including some recent experiments of the junior author (Gardner), using starvation to prolong the time of development.

D.—The senior author has found in connection with other work that at least one inversion in the third chromosome and an echinus deficiency in the first chromosome enhance considerably the expression of plexus.

Discussion.—A discussion of the details will be reserved for the full publication of the experiments. Here only two points may be touched. As a consequence of all recent developments in genetics it will become necessary (as realized both by the senior author and Muller) to decide whether the effects of changes in chromosomal pattern are produced by a change of pattern of the whole chromosome or major parts of it or only by a pattern change within a small section, e.g., the achaete-scute section. Facts of the type reported here tend to show that both types of action exist and that their eventual interrelation will have to be investigated. A fuller discussion of just this point will soon be presented. The other point is this: It has been claimed that curly (*Cy*) can be separated from its inversion, though a *Cy* without the inversion could never be recovered (Bridges). It has further been claimed (Oliver) that a reversion of Plum to wild type has been produced by x-raying, without a change of the chromatin-rearrangement characterizing Plum. Some of the facts communicated here suggest that in both cases something appeared which changed the dominance relations. It has been repeatedly observed by the senior author that in some complicated crosses (not yet analyzed) *Cy*

appeared like ski and even like wild type, but showed *Cy* again if outcrossed. The possibilities of dominance modification have therefore to be considered when a separation of the position-effect from a chromatin-rearrangement is being claimed.

[1] Services rendered by the personnel of Works Progress Administration Official Project No. 465-03-3-192 are hereby acknowledged.

EXTRACTION OF AN EGG MEMBRANE-LYSIN FROM SPERM OF THE GIANT KEYHOLE LIMPET (MEGATHURA CRENULATA)

BY ALBERT TYLER

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Numerous investigators have attempted to extract from spermatozoa a substance capable of activating the unfertilized egg. The results have proved generally negative and where slight effects have been claimed these have not proved repeatable and have been attributed to faulty technique or errors of interpretation (see literature reviews of Loeb,[1] Lillie,[2] Sampson,[3] Morgan,[4] Just[5]). Since the search has generally been directed towards finding a substance of enzymatic nature in the sperm, and since all known enzymes are protein it seems hardly likely that such a substance would enter the egg even if it were present in the sperm extracts employed. What has generally been overlooked in such work is that in normal fertilization the spermatozoön first must enter or at least start to penetrate the egg before the latter is activated. That distinction may be made between penetration and activation of the egg is illustrated by the experiments of Tyler and Schultz[6] on "reversing" fertilization. In those experiments Urechis eggs were treated with acidified sea water within a short time (3 minutes) after insemination. The initial developmental changes reverse as a result of the treatment but the spermatozoön continues its penetration into the egg. Development does not ensue even when the eggs are returned to ordinary sea water. The eggs can be inseminated again with fresh sperm in which case they develop as dispermic eggs. Or they can be activated by a parthenogenetic agent, in which case since a spermatozoön is present in each, they develop as normal monospermic eggs. The distinction between penetration and activation may also be illustrated by cases that have been reported of the entrance of spermatozoa of molluscs or annelids (Kupel-

wieser[7]) or of nemerteans (Chambers[8]) into echinoderm eggs. Here again the eggs are not activated.

In the present work a substance (or substances) has been extracted from sperm which may help to explain the problem of penetration. This material causes the membrane of the limpet egg to dissolve. It is essentially a lytic agent.

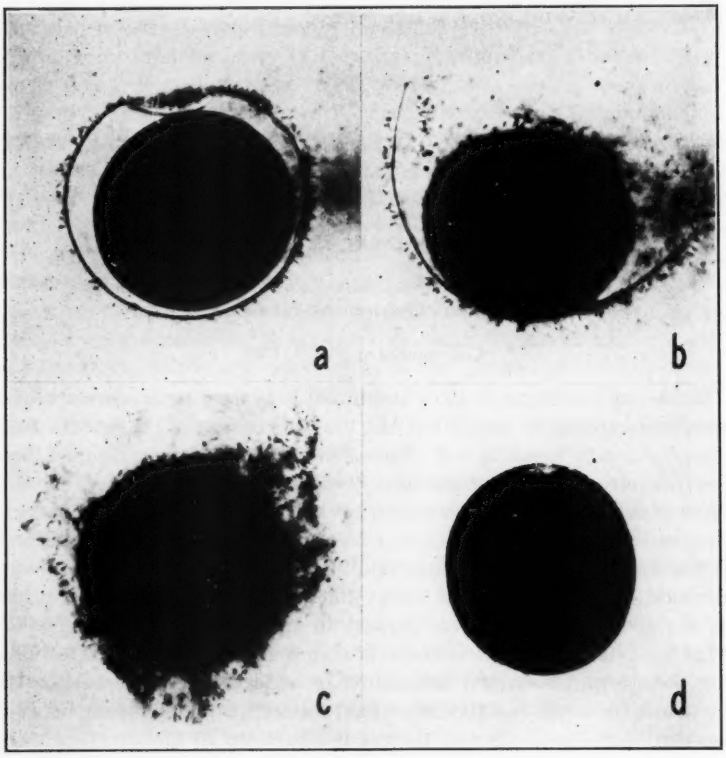

FIGURE 1

Dissolution of membrane of egg of *Megathura crenulata* by means of concentrated sperm. Photomicrographs taken at: *a*, 2 minutes; *b*, 3 minutes; *c*, 4 minutes; *d*, 10 minutes after insemination. Sperm washed off egg in *d*. See text for further details.

The eggs of the giant keyhole limpet, when they are shed, are somewhat irregular in shape and have a rather thick membrane closely adherent to the surface. Outside of the membrane there is a firm jelly layer which is ap-

proximately equal in thickness to one-half of the diameter of the egg (155 μ). As the unfertilized eggs stand in sea water they slowly become spherical in shape and the membrane separates from the surface. After about 20 to 30 minutes in sea water the membrane is fully raised leaving a space equal to about one-tenth of the egg diameter between it and the surface of the egg. The jelly swells and softens in sea water and exceeds the diameter of the egg in thickness.

When the eggs are placed in a concentrated sperm suspension, the membrane is observed to disappear. This effect is illustrated in figure 1. The spermatozoa penetrate the jelly rapidly and adhere to the membrane. When a sufficient number have accumulated the membrane begins to get thinner and lifts off further from the surface of the egg. It then buckles in, particularly in the region where there is the greatest accumulation of sperm (Fig. 1*a*). Presently it vanishes in that region while it continues to expand and thin out elsewhere (Fig. 1*b*). Finally it disappears completely and the sperm accumulate around the egg (Fig. 1*c*). At first it was thought that the large number of spermatozoa around the egg simply obscured the membrane, but after carefully washing off the sperm no trace of the membrane is seen (Fig. 1*d*).

The rate at which this effect occurs depends on the concentration of sperm suspension employed. With very dilute sperm suspensions ($<0.01\%$ of dry sperm) there is no observable effect on the membrane no matter how long the eggs are kept. In a one per cent sperm suspension the membrane disappears after 4 to 5 minutes when fresh eggs are employed. Eggs that have stood for some time in sea water so that the jelly has softened react faster, due very likely to the greater ease with which the spermatozoa reach the membrane.

That the membrane-dissolving agent is in the spermatozoa rather than in the medium was shown by centrifuging a concentrated sperm suspension and testing the supernatant fluid. The latter, even when containing some sperm, failed to cause dissolution of the membrane, while the original sperm suspension (2% of dry sperm) as well as the centrifuged sperm in fresh sea water gave the typical reaction.

The lytic agent may be extracted from a suspension of spermatozoa in sea water simply by freezing it at $-80°$C. for a few minutes and allowing it to thaw at room temperature. The treatment kills the sperm and they become clumped in a gelatinous mass leaving an opalescent solution, free of sperm. This sperm-free extract contains the lytic agent. Washing the sperm mass with sea water furnishes successively smaller amounts of the substance. The yield may be increased by grinding in the frozen state in a ball mill, but this has the objection that increasing amounts of inactive material are thereby likely to be extracted.

Membrane dissolution by means of an extract prepared by simply freez-

ing and thawing is illustrated in figure 2. It proceeds in essentially the same manner as when living spermatozoa are employed. The membrane first increases in diameter, becomes thinner and collapses at the thinnest region (Fig. 2*a*). This continues (Fig. 2*b*) and rather suddenly the membrane disappears, first at the region of initial buckling (Fig. 2*c*) and then

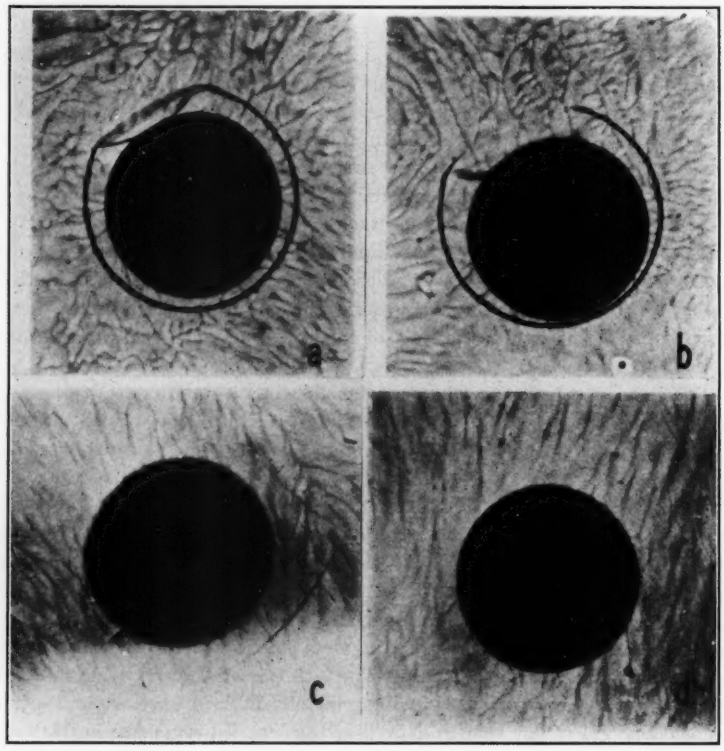

FIGURE 2

Dissolution of membrane of egg of *Megathura crenulata* by means of extract of sperm. Photomicrographs taken at: *a*, 1 minute; *b*, 1 3/4 minutes; *c*, 2 1/2 minutes; *d*, 3 1/4 minutes after addition of extract. See text for further details.

completely (Fig. 2*d*). With an extract of a one per cent sperm suspension, membrane dissolution occurs in three minutes when freshly shed eggs are employed.

The time for complete disappearance can be determined fairly accurately, the average deviation being about 5 per cent of the mean for the different eggs in any test. As the extract is diluted the dissolution time in-

creases. The dissolution time can then be used for bio-assay. The jelly around the egg retards the reaction due undoubtedly to the time required for the substance to reach the membrane. Thus an extract that gives membrane dissolution in two and one-half minutes on eggs with jelly acts in 20 seconds when the jelly is removed. Dilution of the extract increases the time of membrane lysis in an approximately linear manner with jellyless eggs, as the figures in table 1 show.

TABLE 1

DILUTION SERIES

CONCENTRATION OF EXTRACT	TIME FOR MEMBRANE DISSOLUTION EGGS WITH JELLY	JELLYLESS EGGS
1	218 sec.	20 sec.
1/2	242 sec.	39 sec.
1/4	309 sec.	95 sec.
1/8	403 sec.	171 sec.

The lysin is readily inactivated by heat. Some of the heat inactivation data are presented in table 2. Even at as low a temperature as 44°C. most of the activity is soon lost. A low inactivation temperature is characteristic of most lytic agents. That this is lower than generally obtained

TABLE 2

HEAT INACTIVATION OF MEMBRANE-LYSIN

EXPOSURE	PER CENT INACTIVATED AT VARIOUS TEMPERATURES 69°C.	52.5°C.	49°C.	44°C.
1 min.	99.6	75	60	35
2 min.	99.9+	99	98.5	75
4 min.	99.9+	99.5	99.1	80
8 min.	100	99.9+	99.4	85

with hemolysins, for example, is perhaps related to the natural environment (the sea) being at a lower temperature than the mammalian body temperature. The activity of the lysin falls off slowly with time at room temperature; but it may be kept for several weeks at least at 0°C.

The lysin is non-dialyzable through collodion membranes. It can be completely precipitated with ammonium sulphate. The precipitate when washed free of ammonium sulphate on a membrane filter and redissolved in the original volume of sea water has practically the same activity as the original extract.

The precipitated and dialyzed material gives positive Xanthoproteic, Millons and Biuret tests. The Hopkins-Cole test is apparently negative. The material gives a positive test for sulphur. The lytic agent is readily inactivated by crystalline trypsin and crystalline chymotrypsin.[10] It is fairly safe to conclude then that the lysin is a protein.

Alcohol precipitates and at the same time inactivates the lysin. Also in distilled water the lytic action is soon irreversibly (?) lost. This will evi-

dently complicate attempts at further purification and crystallization.

From the eggs of the giant keyhole limpet, fertilizin, a substance capable of causing sperm agglutination (Lillie[2]), is obtained. The fertilizin reaction in the keyhole limpet will be described in a future publication. Frank[9] has recently shown that sea-urchin sperm extracts, prepared by heating, will neutralize the sea-urchin fertilizin. The limpet sperm extract has likewise this property, which was observed before Frank's article appeared. The limpet anti-fertilizin precipitates in saturated ammonium sulphate and is non-dialyzable. This, then, appears to differ in behavior from the sea-urchin anti-fertilizin since Frank reports that he was unable to obtain a precipitate with ammonium sulphate. Heating the limpet sperm extract to 60°C. for two minutes destroys the egg membrane lysin but leaves the anti-fertilizin practically unaltered. There appear then to be at least two distinct substances present in the sperm extract. It is of course possible that both substances are concerned in the lytic action, one of these in the manner of complement. Heating might then destroy the complement and leave the anti-fertilizin.

Work on the purification of the lysin and on its relation to the fertilization process is in progress. The lytic action is also exhibited by abalone (*Haliotis cracherodii*) eggs with extract of abalone sperm. Cross-lysis does not occur between the keyhole limpet and the abalone, nor does cross-fertilization.

The lytic action of the sperm extract is, as far as the present evidence goes, confined to the egg membrane. The membrane of fertilized as well as unfertilized eggs may be dissolved. No particular effect has as yet been noticed on the development of fertilized eggs whose membranes have been dissolved. Freshly shed unfertilized eggs, in which the membrane has been lysed, fertilize as readily as untreated eggs. Unfertilized eggs that have stood some time in sea water so that the membrane is well separated from the surface fertilize more readily when the membrane is lysed. A rapid falling off of fertilizability after the eggs have been shed into water is characteristic of many other animals (e.g., fish and amphibia). It may be suggested that the effect is due to elevation of the vitelline membrane so that the spermatozoa are incapable of making immediate contact with the egg surface. It is, in fact, a generally accepted view that final incorporation of the spermatozoön is effected by the egg entrance cone.

Loeb[1] developed a theory of fertilization and parthenogenesis in which he postulated the existence of two substances; one, a specific cytolysin which if allowed to act unchecked leads to destruction of the egg; the other, a corrective agent which limits the destructive influence of the lysin. It should perhaps be emphasized that there is nothing in the present work to support Loeb's view, particularly as his theory concerns the activation of the egg. The lytic agent demonstrated to be present in the limpet and

abalone sperm appears simply to be concerned in the penetration part of the fertilization process.

Summary.—A lytic agent having the property of dissolving the egg membrane has been extracted from sperm of the giant keyhole limpet (*Megathura crenulata*). The lysin is non-dialyzable and readily inactivated by heat. It precipitates upon saturation of the extract with ammonium sulphate. The precipitate gives the usual protein tests and pure proteinases inactivate the lytic agent. Abalone (*Haliotis cracherodii*) sperm also yield a lysin which acts on the eggs of the same species. Cross-lysis between limpet and abalone does not occur; nor does cross-fertilization. Limpet sperm extracts also contain a substance (anti-fertilizin) capable of neutralizing the sperm agglutinin (fertilizin) obtained from eggs. Heat inactivation of the lysin leaves the anti-fertilizin unaffected.

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[9] J. A. Frank, *Biol. Bull.*, **76,** 190 (1939).

[10] The crystalline proteinases were very kindly supplied by Dr. J. H. Northrop.

THE WOUND HORMONES OF PLANTS II. THE ISOLATION OF A CRYSTALLINE ACTIVE SUBSTANCE

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Introduction.—The existence of plant "wound hormones" has been recognized since the formulation of the wound hormone concept by Wiesner[1] and the demonstration by Haberlandt[2] of the diffusible nature of the active principles. It was shown by these early investigators that there are formed or liberated at injured surfaces of plant tissues water-soluble substances which are capable of evoking renewed growth activity in mature uninjured cells or tissues. In the present paper the isolation from plant

material of a crystalline substance possessing typical "wound hormone" activity will be described.

Materials and Methods.—Numerous methods for the demonstration of wound hormone activity have been suggested.[3] For the present work the parenchymatous mesocarp lining the seed chambers of the string bean pod has been used as the test material. It has been found by Wehnelt[4] that if drops containing the water extract of bean are placed on the uninjured cells of the mesocarp, a vigorous proliferation and elongation of the underlying cells results, whereas drops of pure water are without such an effect. A detailed description of the adaptation of this reaction to the quantitative estimation of wound hormone activity has been presented elsewhere.[5] Whereas others have attempted to utilize as a measure of wound hormone activity the frequency of cell divisions in the resulting intumescences, we have utilized the total volume of new tissue formed. This volume, as determined by the height of the intumescence, is not only linearly proportional to the concentration of wound hormone solution applied, but is also easily determined and well adapted to large scale routine bioassays. In brief, the method is as follows: Young pods of Kentucky Wonder string bean (*Phaseolus vulgaris*) are slit longitudinally, the seeds removed, sections containing the seed chamber excised and arranged in Petri dishes, 30 sections per dish. Uniform drops (0.01 cc.) of each solution to be tested are placed on 20 sections from 20 individual beans. Each of the 20 individual beans is also treated with two concentrations of a standard bean extract.[6] This solution of standard activity is tested daily on every set of beans and activities of the unknowns referred to it. The test beans are then incubated for 48 hours at 25°C. At the end of this time cross-sections through the seed chamber and intumescence are cut and the total height of each intumescence measured under a low-power binocular equipped with an eyepiece micrometer. It has been found readily practicable to assay thus 30 solutions per day, each solution tested on 20 beans, together with the above-mentioned standard solutions.

In following wound hormone activity through a series of fractionations of the initial crude material it has been found desirable in the present work to make use of an arbitrary "unit" of activity. A *unit* of wound hormone activity will be defined as that amount which when present in 1 cc. of solution will, when this solution is tested, give a reaction equal to that of the standard bean extract.

During the course of the procedure outlined below for the isolation of a crystalline wound hormone it was found that there are one or more "cofactors," themselves inactive or essentially so, which have, however, the property of greatly increasing the potency of the more active fraction. This can be made clear by an example of the distribution of activities before and after fractionation of concentrated extract with chloroform, as is

shown in table 1. Only a small amount of activity remained in the water layer after extraction with chloroform. The chloroform-extractable material contained, when tested, alone, only one-fourth of the total amount of activity present in the starting material. If, however, the two fractions were recombined the mixture was found to possess a total number of units of activity essentially equal to that of the starting material. This behavior would be in accordance with the view stated above that there remained in the chloroform-insoluble material a substance (or substances) inactive in itself but capable of enhancing the activity of the chloroform-soluble fraction. A similar behavior was noted at other stages of the fractionation, notably at steps 2 and 3 below. For this reason, in the experiments reported here the active material was invariably tested in the presence of a mixture of two of the discarded inactive fractions, including the acetone and ethyl acetate insoluble portions.[7]

TABLE 1

DISTRIBUTION OF ACTIVE MATERIALS BETWEEN $CHCl_3$ AND WATER

	MG.	UNITS/GM.	TOTAL UNITS
Starting material	201	170	34.2
$CHCl_3$ extract (continuous extraction)	16	500	8.0
Water layer	185	30	5.5
Combination	201	150	30.2

Procedure. 1. One hundred pounds of fresh bean pods were ground and immediately extracted twice with water at 50–60°C. for one-half hour. The suspension was filtered and the clear solution (about 100 liters) was evaporated at once under reduced pressure under nitrogen at a bath temperature of 60°C. The evaporation was continued until a thick syrup (85% solids) was obtained.

Yield—2750 gm. 48 units/gm. 132,000 units

2. The syrup thus obtained was acidified with concentrated hydrochloric acid to pH 2 and extracted repeatedly with acetone. The bulk of the activity was found in the acetone layer.

a. Acetone layer	554 gm.	170 units/gm.	94,000 units
b. Water layer	2200 gm.	0 unit/gm.	0 unit
c. Combination	2750 gm.	48 units/gm.	132,000 units

3. The acetone was evaporated off in nitrogen at 60°C. and the residue taken up in water (2000 cc.) and adjusted to pH 2. This solution was exhaustively extracted with ethyl acetate. All tests were carried out in cofactor mixture.

a. Ethyl acetate layer	67.4 gm.	2000 units/gm.	135,000 units
b. Water layer	487 gm.	35 units/gm.	17,000 units

4. The ethyl acetate was evaporated as before and the oily residue dissolved in dilute sodium hydroxide, adjusted to pH 10–11 and extracted with chloroform. The water layer was then acidified to pH 2 and extracted again with chloroform. At this point an oil material separated which was insoluble in both chloroform and water. In view of its comparatively low activity this was set aside. All tests were carried out in co-factor mixture.

a. Chloroform from alkaline solution, 4.0 gm.; 650 units/gm.; 2600 units
b. Chloroform extract from acid solution, 7.3 gm.; 6800 units/gm.; 50,000 units
c. Oily material, 8.2 gm.; 1450 units/gm.; 12,000 units
d. Water layer, 39 gm.; 0 unit/gm.; 0 unit

5. Fraction 4*b* (which contains organic acids) was dissolved in 0.1 normal sodium hydroxide after evaporation of the chloroform and a solution of barium chloride added. There resulted a dark, oily precipitate which was separated by centrifuging and washed with water. Both filtrate and precipitate were treated with dilute hydrochloric acid and the acidified solutions exhaustively extracted with ethyl acetate. The regenerated precipitate was a very dark, oily material and the filtrate was a light brown viscous oil which partially crystallized on standing.

a. Insoluble barium salts	4.2 gm.	2200 units/gm.	9000 units
b. Soluble barium salts	3.1 gm.	2900 units/gm.	9000 units

6. The crystals of 5*b* were separated by the addition of cold peroxide-free isopropyl ether and filtration of the thick syrup on a sintered glass filter. The crystalline mass thus obtained was pressed on a porous plate and then recrystallized from absolute alcohol to constant melting-point and activity. In this way there were obtained 18 milligrams of crystalline material, m. p. 165.5–166°. By petroleum ether extraction of the oily mother liquors, further active fractions were obtained including an additional small amount of this crystalline material with identical properties and activity.

Analysis.[8] Found: % C, 63.37; % H, 9.01. Calculated for $C_{12}H_{22}O_4$: % C, 63.15; % H, 8.77. Calculated molecular weight, 228. Equivalent weight by titration, 118.

Preparation of the Methyl Ester.—Fifteen milligrams of crystalline material, m. p. 163–164°, recovered from mother liquors of recrystallization were dissolved in ether and treated with an excess of an ethereal diazomethane solution at 0°. After standing for an hour, the ether was distilled off and the residual oil distilled *in vacuo*. At 140°C. bath temperature a nearly colorless oil distilled which crystallized on cooling. The crude ma-

terial (m. p. 29–31°) was recrystallized from a mixture of ether and petroleum ether to a constant m. p. of 30–31°.

Analysis. Found: % C, 65.21; % H, 9.18. Calculated for $C_{14}H_{24}O_4$: % C, 65.55; % H, 9.40. Calculated molecular weight, 256. Molecular weight, by m. p. depression in camphor, 267.

On hydrolysis in alcoholic KOH the acidic starting material was regenerated, as judged by m. p. and activity.

Proof of Purity.—In order to ascertain whether the activity was due to the crystalline material itself or to small amounts of adsorbed impurities, 15 mg. of the substance were subjected to chromatographic analysis on aluminum oxide from $CHCl_3$ solution. No alterations in melting point or activity of the material could be effected in this way.

Nine milligrams of crystalline material were sublimed *in vacuo* (0.1 mm. of Hg) at a bath temperature of 160°C. The sublimate possessed the same m. p. and activity as the starting material.

Activity of the Pure Material.—The pure crystalline material has been assayed for its activity 60 times in the bean test in the presence of the standard co-factor mixture. The average activity has been found to be 4800 units per gm. or about 100 times that of the starting material. In the absence of the co-factor mixture the activity of the crystalline substance varied greatly in successive daily tests, with a maximum of 4800 units/gm., a minimum of 0 unit/gm. (one test) and a mean activity of 2700 units/gm. The fluctuations in activity of the pure material when tested alone would be in accordance with the hypothesis that co-factors are to some extent present in the test beans themselves, but that their amount varies from day to day due to unidentified and uncontrolled variations in environmental circumstances.

Numerous pure substances have been tested for co-factor activity in the presence of the crystalline factor. Attention was first given to sugars and amino acids since these were found to constitute a large portion of the initial water extract and were also known to constitute a considerable part of the acetone and ethyl acetate insoluble fractions. When the crystalline factor is tested together with 0.5% sucrose (which is itself completely inactive) the activity is increased from 4800 to 6600 units/gm. Of the amino acids tested glutamic acid was found to exert a marked co-factor activity. Glutamic acid at high concentrations is itself slightly active in the standard bean test (approximately 100 units/gm.). At a concentration of 0.25%, however, the intumescence evoked by glutamic acid is sufficiently small to be disregarded. When the crystalline factor is tested in the presence of 0.25% glutamic acid the activity of the former is enhanced to approximately 45,000 units/gm., or almost ten times that obtained with the standard co-factor mixture. The crystalline factor at a concentration of 10 gamma per

cc. and in the presence of 0.25% glutamic acid can evoke intumescences even higher than those evoked by the standard bean extract.

Discussion.—The crystalline wound hormone possesses detectable activity at a concentration of 10 mg. per liter in the presence of 0.25% glutamic acid. At this concentration, 0.1 gamma would be present in each of the 0.01 cc. drops which are applied in the standard bean test. This substance is thus comparable in potency to other of the physiologically active substances.

The substance isolated can account for at most 10% of the total wound hormone activity present in the initial extract. This may be attributable either to the presence of other as yet unrecognized co-factors in the initial extract, or to the presence in this extract of still further highly active substances. With relation to the first possibility, there is a highly labile co-factor, itself inactive, present in the water layer of step 4*d*. It has been impracticable because of its instability to incorporate this co-factor in the standard co-factor mixture. Because of its high activity the discarded fraction (5*a*) from the barium salt separation could not be incorporated into the standard co-factor mixture. In these two fractions, then, there may well be inactive co-factors capable of increasing still further the activity of the substance which has been isolated.

With relation to the second possibility, that there may be still further highly active wound hormones present in the initial extract, it has been shown that glutamic acid possesses real although low activity. The non-crystalline material (traumatin) whose isolation has been previously reported[5] also appears to differ both in chemical properties and in activity from the crystalline substance reported here. The nature of other possible active materials will be the subject of future communications.

It is of interest to know in how far the present crystalline material functions in the regulation of cell division. Preliminary histological investigations have shown that under the influence of the pure substance, in the absence of added co-factors, both cell division and cell enlargement take place in the tissue of the bean mesocarp.

Summary.—The isolation from fresh beans of a crystalline substance possessing typical wound hormone activity has been described. The substance, a dibasic acid, the analysis of which corresponds to $C_{12}H_{20}O_4$, is capable of evoking renewed cell division and cell extension activity in the mature parenchymatous cells which compose the mesocarp of the string bean pod.

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[6] For the preparation of the standard bean extract, 200 mg. of a dried, finely ground bean powder are boiled for 1 minute in 10 cc. of water, filtered and the filtrate made up to 10 cc. The resulting solution contains approximately 10 mg. of solid material per cc. By testing both this 1% solution and a solution diluted to 0.5%, the slope of the intumescence height vs. concentration curve is obtained daily. One cubic centimeter of the standard 1% solution is defined as containing 1 unit of activity. The dried bean powder has been used over a period of 2 years without detectable loss of activity.

[7] The ethyl acetate insoluble fraction of step 3*b* possessed a small activity. This activity was completely removed by further extraction with ethyl acetate and the deactivated portion used as a component of the standard co-factor mixture. The water layer of step 2*b* and the water layer of step 3*b* were mixed in the proportions of 4.5 to 1 and the resulting solution used in a final concentration of 1% as a diluting medium for the unknown solutions.

[8] Microanalyses by G. A. Swinehart.

THE CHEMICAL INDUCTION OF GENETIC CHANGES IN FUNGI

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Biochemical and technical studies of Aspergilli began about the middle of the 19th century. By 1930, Tamiya and Morita cited 2424 numbers in their bibliography of the genus, and many have been added since. Of all the molds which have caught man's fancy as experimental material the black Aspergilli—under the name *A. niger* van Tieghem—have had the greatest share of attention starting with the basic assumption that they were a single species, hence easy to identify.

A few outstanding references to *A. niger* will show some of the reasons for our study. As far back as 1867, van Tieghem described the use of a black Aspergillus in fermenting tannins toward gallic acid. That factory process is still in use. In 1891 Wehmer showed that his strain produced oxalic acid from sugars. In 1916, Thom and Currie tested twenty such strains for oxalic acid production and found variations as great as ten to one in the amount of oxalic acid produced by different strains. From this series, Currie selected one as a citric acid fermenter and developed production upon a factory scale which is said to have reached half of the citric acid used in the United States. The basis of this fermentation at that time was granulated sugar and the agent was a black Aspergillus originally iso-

lated from a sample of moldy butter. We are vaguely informed that the same factory now uses a more efficient black Aspergillus to do the same amount of work with less and cheaper material. Another group is making gluconic acid by a similar process with one of the same organisms.

There is not time here to discuss other uses of *A. niger* or the technical applications of other molds. For our purpose, certain items are important. Variants from van Tieghem's original description of *A. niger* have been found occasionally in nature. Collectors have accumulated a considerable number of them in half a century. Strains are found with big or little fruiting heads, with spores twice or three times the size in the type description, with long stalks or short stalks, with sclerotia or producing striking color differences. Mosseray working wth Biourge's collection of 63 strains, described forty species which appeared to him to show stable differences warranting separation.

Under rigidly standardized culture conditions as handled by Biourge, the differences in those forty species are tangible, but these variants fall automatically into several very closely related series. An offer to find 500 more to test the taxonomist's scheme of classification was not accepted. How are we to interpret these variations? How are we to identify the organisms important to us? For the most part, we have been compelled to obtain a valuable organism by quantitative testing of the strains available, and to keep that strain in pure culture because if once lost, there is no routine means of picking it again from a host of strains differing little in appearance.

The sporadic occurrence of striking variations among black Aspergilli in nature raises the question whether they arise from internal or external causes. Most of the differences seen are minor and quantitative (more, or less, of this or that characteristic), but recently some very striking mutants have been discovered. The Yuills have described one of them as the new genus Cladocera. Another appeared in a factory in which it was carefully isolated and sent to us, with inquiry as to its nature and its menace, if any, to operation.

In another group of Aspergilli and without technical significance apparently, a similar series of variants has appeared which bridges the gap from *Aspergillus sydowi* to *Penicillium restrictum*. Thus what we thought were generic lines seem to have disappeared in this one case. Among hundreds of strains which appear in the routine operation of a laboratory dealing with decomposing organic materials, such variants are not numerous at any time but have added appreciable numbers to our collection in thirty-five years.

The possibility that such changes, few under field conditions, could be induced to increase in frequency by chemical treatment after the manner used by our phanerogamic neighbors who apply colchicine or some other

agent, was alluring. The selection of a basic strain for experiment became necessary. We specified (1) an organism whose history had been known for a considerable time, (2) it should be as free as possible from spontaneous variation, (3) it should be readily grown under laboratory conditions.

Many years ago, Schiemann had produced three mutants of *Aspergillus niger* by chemical means. Two of them are still in our culture collection, unchanged after twenty-five years, but they differ from type only by differences in the quantity of color produced. Other contrasts were not found. Currie had maintained his citric acid organism with a 50% yield of citric acid from sugar consumed, for twenty years, but while we had the original, we did not have the organism as at present in culture at the factory. On the other hand, Steinberg's strain meticulously studied in 1917 to 1919 was still in our possession and had proved stable in morphology. It had been retested for reactions carefully established twenty years before and found essentially unchanged. This organism was selected for primary experiments. For checking purposes, the two organisms selected were (1) a strain (No. 126) of *Aspergillus amstelodami* Mangin which had proved stable in our cultures for thirty years, and (2) *Penicillium caseicolum*, apparently the type strain from the Bainier collection which had been under observation here for twenty years against strains of the same species in our possession for thirty-five years. It was believed that these species might yield results of interest.

It should be noted at the outset that in experiments with *Aspergillus niger* or *Penicillium caseicolum*, we are not dealing with a sex problem. No so-called perfect form has been found for either aggregate. *Aspergillus amstelodami* in contrast, regularly and quickly produces ascospores.

A survey of possible methods of work was necessary. The molds present two possibilities for attack: (1) The spores or propagative cells may be treated, then inoculated into favorable substrata. This expedient was tried with *Aspergillus niger* by transferring masses of spores to test solutions from which transfers were made to culture substrata. In one experiment covering a period of sixteen weeks with tests at frequent intervals, only the usual type of growth was obtained. The spores retained their vitality but showed no variant tendencies which could be called mutation. (2) The alternative was to put the test substance in solution into the culture substratum and grow new mold colonies with the vegetative mycelium in contact with the stimulant. From such colonies either spores or vegetative mycelia could be transferred to normal substrata. If variation or mutation had taken place, the changes would appear in the new colony and could be carried through subsequent generations. The findings reported here were obtained by such manipulation. Large scale platings of the *Aspergillus niger* used in most of the work disclosed no contaminating species.

A total of 37 dyes and biological stains were tested in culture in agar slants and petri dishes, and with solution, using *Aspergillus niger*, *Aspergillus amstelodami* and *Penicillium caseicolum* under various conditions. Variants were obtained with *A. niger* on agar slants using aniline blue, acridine yellow, methylene blue and methylene violet. These strains were vigorous growers with mycelia of bright yellow color and retarded sporulation. The results could not be duplicated at will, either because the inciting cause if evident was overlooked, or because such sporadic variations arise for causes internal to the organism but not measurable by the operator.

Acridone, a compound formed from salicylanilide by heat, appeared to be responsible for a non-perithecial form of *A. amstelodami*.

Iodine produced striking changes in the colony developed but the few spores produced grew into typical *A. niger* colonies. Such changes were toxic effects on the single vegetative generation.

Tests with *A. niger* in solution cultures with aniline blue, methylene blue, methylene violet, acridine yellow, colchicine and acenaphthene gave negative results even with highly toxic concentrations (2%). Plates saturated with these materials gave negative results also.

A total of thirty-one miscellaneous compounds including colchicine and acenaphthene were tested under the same conditions. No formation of variants attributable to treatment was observed.

A total of eighty-five phenanthrene derivatives were tested with nutrient solution, but without result with *A. niger*.

Consistent results, readily duplicated were obtained with a mannitol-nitrite solution of the following composition:

Water (distilled in Pyrex still)	1000.0	gm.
d-Mannitol	50.0	gm.
$NaNO_2$	2.0	gm.
K_2HPO_4	0.35	gm.
$MgSO_4.7H_2O$	0.25	gm.
$FeSO_4.7H_2O$	0.001	gm.
$ZnSO_4.7H_2O$	0.00088	gm.
$CuSO_4.5H_2O$	0.00020	gm.
$MnSO_4.4H_2O$	0.00012	gm.
$NaMoO_4.2H_2O$	0.00005	gm.
H_2SO_4 to pH 4		

This solution has been used by Steinberg in his studies of the relation of nitrogen to the trace elements. It is referred to commonly as the "dibasic optimum" solution in which mannitol has been substituted for sucrose, sodium nitrite for ammonium nitrate and sulphuric acid added to produce any desired acidity. In routine studies, the trace elements can probably be ignored.

Inoculations into three of four 200 cc. flasks each containing 50 cc. of this solution were massive, and the cultures were permitted to grow for a minimum of one month. Transfers from selected portions of these cultures were made to Czapek slants at intervals. While active growth usually ceases in four weeks, new growing areas sometimes develop in old cultures. Transfers from such regions usually produce variants. Concentrations of nitrite and acidity need to be adjusted to the specific organism for best results probably.

Three series of tests were performed with the mannitol-nitrate solution.

Series 1. With *A. niger*, produced yellow woolly variant colonies.

Series 2. With *A. niger*, reproduced the same variants as Series 1.
With *A. amstelodami*, produced masses of mycelium, abundant green heads but reduced perithecia to very few.
With *Penicillium caseicolum*, produced no variants.

Series 3. With *A. niger*, continued the production of the same types of variants.
With *A. amstelodami*, produced no variants determinable thus far.
With *P. caseicolum*, produced no variants.

Series 3 was made with a concentration of nitrite great enough to stop the production of variants in *A. amstelodami*, and reduce its development. In contrast, *A. niger* produced variation to a greater degree with the increased nitrite.

Differences between the original *A. niger* or *A. amstelodami* strain and the variants produced were readily discerned with the naked eye. Detailed examination with high magnification produced confirmatory evidence to a surprising degree. It has been possible in this way to find in the variants from Steinberg's strain structures figured in detail in Mosseray's monographic study of Biourge's black Aspergilli. Biourge's collection of Aspergilli included 63 strains selected over many years from the miscellaneous material reaching him from the Belgian colonies, especially in Africa, and from correspondents.

The group of experiments may be summarized as follows:

(1) Under nitrite stimulation, the Steinberg Aspergillus produced certain of the same types of variants in successive experiments, each beginning with the unchanged stock culture.

(2) The changes in morphology observed include increased vegetative mycelium, increased production of yellow to orange color in the hyphae, reduction in conidia producing apparatus in the form of diminutive stalks, reduced size of head, elimination or reduction of the primary sterigmata, great reduction in the numbers of spore producing cells and the production of comparatively few spores.

(3) Conidia or spores when present have been indistinguishable from the type, and viable in these experiments.

(4) Variants produced under controlled conditions have essentially duplicated others discovered sporadically in nature. It appears that this strain of black Aspergillus tends to vary in fairly definite directions.

A similar series of organisms from the field showing reduction from the typical Aspergillus head as seen in *A. sydowi*, to a form indistinguishable from or identical with *Penicillium restrictum* Gilman and Abbott, has been brought together within the last two years.

In a later series of experiments our strain No. 126 of *Aspergillus amstelodami* was included. This organism produces perithecia and ascospores abundantly with a moderate quantity of green heads bearing asexual spores or conidia. The colony picture had remained fairly dependable in stock transfers over a period of thirty years. Consistently with the *A. niger* experiments the nitrite solution caused reduction of certain phases and accentuation of others. Summarized the changes observed were:

(1) The number of perithecia dropped from excessively abundant to very few and differently placed; the accessory or green conidial phase is increased along with increase of the vegetative mycelium. In other words, reduction began with the complex form of fruiting and accentuated the simpler form.

(2) Ascospores and conidia when present show the marks characteristic of the species.

(3) *Penicillium caseicolum* has refused to respond to stimulants thus far applied.

This report is only a beginning. No cytological studies have been made. Only one strain of black Aspergillus out of many hundreds has been tested. Only one active stimulant has been found. It is probable that other agents might turn the tendency to vary into other directions morphologically or physiologically. It is perhaps significant that after trying to accomplish this purpose through the use of complex organic compounds, the substance found to be most effective was an inorganic and simple one which is reported as present in green plants as well as fungi. Interference in the normal reduction of nitrate through the presence of toxic substances might therefore possibly lead to an accumulation of sufficient nitrite to induce similar genetic variations. The quantity of nitrite required is quite small and equivalent to not over half the nitrogen required for maximum growth. Future investigations should disclose also whether these results are of general application and have a larger significance than as merely an aid in the artificial production of variants. The type of sterility produced in many of these variants appears to disclose a loss of power to differentiate, which is perhaps equivalent to a continuance of embryonic conditions, represented in the molds by purely vegetative growth. As an

hypothesis in explanation, the effect of nitrite upon these fungi is apparently due to a reduction in free amino nitrogen in some protein of the mold.

These experiments seem to give a clue to the origin of groups of strains stable enough to remain taxonomically separable for long periods in standard culture substrata, but in which spore characters are practically identical. It would appear that further experimental work upon similar lines might furnish means of interpreting other groups, and means of producing variations showing useful biochemical activity.

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POLYSOMATY IN CUCUMIS MELO

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The term *polysomaty* was applied by Langlet in 1927 to the condition of those cells (or of their nuclei) in the somatic tissues of a plant which contain multiples of the typical chromosome number. Those nuclei with the typical (commonly diploid) number are *monosomatic;* those with multiples of this number are *disomatic, tetrasomatic,* etc. The occurrence of polysomatic, especially of disomatic, cells in root tips has long been known. Their appearance can be induced by various agencies which check nuclear or cell division. Disomaty has been reported to be caused by nuclear fusion. Disomaty occurs also "spontaneously," that is, from unknown causes.

Disomatic and tetrasomatic cells have been reported as occurring frequently and regularly in the root tips of various members of the Chenopodiaceae. They are observed chiefly in the periblem in the older part of the meristematic region. *Spinacia oleracea* is the species most studied in this connection. One species of a distinct family, *Cannabis sativa,* shows the same condition. In equatorial plates in these polysomatic cells the chromosomes are often conspicuously paired; in others some chromo-

somes are paired; in still others the individual chromosomes are as much separated as in ordinary monosomatic plates. Lorz[1] and Wulff[2] have summarized the earlier work in this field.

The somatic chromosome number ($2n$) in *Cucumis Melo* L. is reported as 24 by Kozhukhow[3] and Whitaker.[4] I have found the same number in Honey Dew, Honey Rock, Bender's Surprise and Hearts of Gold varieties of this species.

Kozhukhow[3,5] observed occasional disomatic cells in root tips of *Cucumis sativus*, *C. Melo*, *Cucurbita Pepo* and *Citrullus vulgaris*. He figures disomatic equatorial plates in each of these species except *Cucumis Melo*, and concludes that the Cucurbitaceae in general are inclined to form disomatic nuclei. He finds, however, that in *C. sativus* disomatic nuclei seldom occur, and then only in the periblem near the region of growth. The chromosomes of disomatic nuclei were found to be paired only in cells of the oldest part of the meristem.

Differently from Kozhukhow, the writer finds polysomaty to be not merely occasional but of regular occurrence in the periblem of root tips of each of the four previously mentioned varieties of *Cucumis Melo*. The conditions here thus closely resemble those already described in various Chenopodiaceae.

In cross sections of the primary root as far back as mitotic figures are to be found, the disomatic cells are fairly abundant throughout the periblem. No localized polysomatic sectors have been observed; it is perhaps for this reason that the roots maintain a regularly cylindrical shape. Polysomaty is of rare occurrence in the plerome; only four cases have been observed in this part of the root.

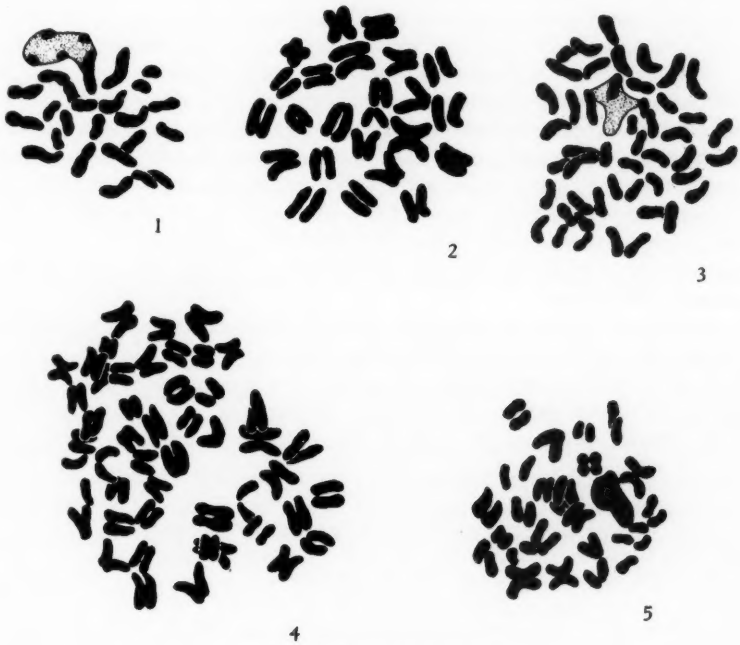
Division figures are first observed in typical primary roots at a distance of approximately 144 μ from the extreme tip. These are monosomatic (Fig. 1). The first disomatic figures (Figs. 2, 3) appear approximately 288 μ from the tip; their frequency increases rapidly toward the older part of the root. A few tetrasomatic equatorial plates (with 96 chromosomes; Fig. 4) have been found in the oldest portion of the meristematic region.

Disomaty and tetrasomaty have been observed in secondary roots, but less frequently than in the primary root. Polysomatic prophases and equatorial plates, both with partial and with complete pairing of the chromosomes, have been seen, although not frequently, in the epidermis and hypodermis of the stem tip (Fig. 5) and of leaf primordia.

Di- and tetrasomatic nuclei are consistently much larger than monosomatic nuclei. Disomatic periblem cells near the root tips, and the rare plerome cells which contain disomatic nuclei, are not highly vacuolated, as are those in the periblem of the posterior meristematic region containing a greater proportion of disomatic cells.

The chromosomes of *C. Melo*, while small, differ sufficiently in length for

certain ones to be recognizable. In monosomatic equatorial plates (Fig. 1) there is no conspicuous tendency for homologous chromosomes to pair. In disomatic plates all degrees of pairing are observed. In some cases one or a few pairs are evident (Fig. 3), all the other chromosomes being more or less scattered; at the other extreme are plates in which all the chromosomes are closely paired (Fig. 2). The members of each pair are in all cases similar in size. The same conditions as to pairing are observable in median and late prophases of disomatic nuclei.



EXPLANATION OF PLATE

Figures 1–4. Somatic equatorial plates from the periblem in primary roots of *Cucumis Melo* variety Honey Dew: 1, monosomatic equatorial plate with 24 chromosomes and nucleolus; 2, disomatic equatorial plate with 48 chromosomes arranged in 24 pairs; 3, disomatic equatorial plate with 48 chromosomes more or less scattered; 4, tetrasomatic equatorial plate with 96 chromosomes arranged in 48 pairs, reconstructed from two serial sections. Figure 5. Disomatic equatorial plate with nucleolus and 48 partially paired chromosomes from hypodermis of the stem tip. All figures × 3650.

A di- or tetrasomatic nucleus contains either a single large nucleolus, often lobed, or two or three smaller nucleoli. A monosomatic nucleus has consistently one nucleolus. The chromosomes attached to the nucleolus

(probably satellite chromosomes) remain so connected, in many cases, until the equatorial-plate stage. In a lateral view of the equatorial plate, the nucleolus appears as a pyriform structure. In some cases it seems to be attached to the chromosomes at one side of the plate, but looped over so that the greater part of it lies on the opposite side. In lateral views of certain anaphases there appears at one end of the cell a chromatic body, evidently the nucleolus, surrounded by a hyaline region.

A few apparently binucleate cells were observed in the periblem and dermatogen both in longitudinal and in cross sections. The origin of polysomaty is held by some workers to be due to the fusion of nuclei in binucleate cells; others contend that these binucleate cells are too rare to account for polysomaty, and that the fusion of resting nuclei cannot account for the closely paired arrangement of the chromosomes.

Summary.—Polysomaty is a regular occurrence in the periblem of primary and secondary roots in four varieties of *Cucumis Melo*. It is rare in the plerome. Disomaty is the more frequent condition, but some tetrasomatic cells appear.

There is an increase in frequency of polysomatic nuclei from the younger toward the older portion of the meristematic region.

In some polysomatic nuclei all the chromosomes are paired; in some, pairing is incomplete; in others there is no pairing.

Polysomaty occurs in the stem tip and in leaf primordia.

[1] A. Lorz, *Cytologia*, **8**, 241–276 (1937).
[2] H. D.Wulff, *Planta*, **26**, 275–290 (1937).
[3] Z. A. Kozhukhow, *Bull. Appl. Bot. Pl. Breeding*, **14**, 89–96 (1925).
[4] T. W. Whitaker, *Amer. Jour. Bot.*, **17**, 1033–1040 (1930).
[5] Z. A. Kozhukhow, *Angew. Bot.*, **10**, 140–148 (1928).

PRODUCTION OF ATOMIC RAYS AND OF COSMIC RAYS IN SUPERNOVAE

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A. Supernovae.—Several years ago, Baade and I made the following suggestions:[1]

(1) A class of very rare temporary stars exists, some of whose major physical properties are similar to those of common novae, but in a superlative degree. We proposed that the new stars might appropriately be called supernovae.

(2) Supernovae are an origin of the cosmic rays.

Our first problem obviously was to locate as many *active* supernovae as possible in order to prove the correctness of assertion (1). Through the discovery of nine supernovae[2] with the Schmidt Telescope on Palomar Mountain, the solution of this problem has now reached a stage of completeness which is sufficient to allow us to turn our attention to assertion (2). We therefore proceed to outline some of the general processes which presumably take place in supernovae and which are capable of producing *cosmic* rays as well as *atomic* rays of high energy.

B. Atomic Rays Ejected in a Supernova Outburst.—We may assume that, similar to the well-known phenomena which take place in common novae, gaseous clouds of considerable mass are ejected from supernovae. An atom of the mass m will have the kinetic energy

$$\epsilon_k = m\bar{v}^2/2 = kmc^2 \qquad (1)$$

where $\bar{v}$ is the average velocity of the ejected gas clouds, and c is the velocity of light.

Since it has not yet been possible to interpret the spectra of supernovae, we do not know the exact numerical values which the constant k assumes for individual supernovae. A number of reasons may be advanced, however, which indicate that k for a supernova is at least a hundred times larger than for a common nova. For instance, it was found by Minkowski[3] that the permanent features in the spectra of four recent bright supernovae were subject to a general progressive red-shift, a shift which over the period of one year corresponds to a relative change in wave-length λ of about

$$\Delta\lambda/\lambda \cong 0.05. \qquad (2)$$

This red-shift, so far, leaves us with the choice of two interpretations. If real, it may indicate

(*a*) A progressive change in Doppler displacement, corresponding to a change of the actual velocities of the ejected atoms.

(*b*) A progressive gravitational red-shift caused by the continuous contraction of the stellar remnant of a supernova into a highly collapsed star.[4]

In case (*a*) we conclude from (2) that, in the early stages of a supernova, the velocity of ejection $\bar{v}$ must be

$$\bar{v} \geqslant 0.05c = 15000\ km/\text{sec}. \qquad (3)$$

with a resulting value k_1 of k,

$$k_1 \geqslant 1.3 \times 10^{-3}. \qquad (4)$$

In case (*b*) we conclude from (2) that the gravitational potential on the

surface of the stellar remnant of a supernova, about one year after maximum, is

$$\varphi = -c^2\Delta\lambda/\lambda = -0.05c^2. \tag{5}$$

The average potential energy ϵ_p of a particle of mass m in the stellar remnant therefore is

$$\epsilon_p < -0.05mc^2. \tag{6}$$

Consequently, during the contraction of the stellar remnant, whose mass is M, the total gravitational energy

$$\mathfrak{E}_t > 0.05Mc^2 \tag{7}$$

has been liberated in form of radiation, kinetic energy of the ejected gas clouds, etc. I have recently shown[4] that the total energy $\mathfrak{E}_{vis}$ liberated in form of visible light (in case (*b*)) constitutes but a small fraction of $\mathfrak{E}_t$. A major part of $\mathfrak{E}_t$ is available to endow every ejected particle of mass m with a kinetic energy of the order

$$\epsilon_k \cong 0.05mc^2 \tag{8}$$

which corresponds to a value k_2 of k, where

$$k_2 \cong 5 \times 10^{-2}. \tag{9}$$

Finally, from simple considerations[5] regarding the light pressure on the ejected gas clouds, an approximate relation may be derived connecting the luminosity of a nova at maximum brightness with the average velocity of expansion $\bar{v}$ of the ejected gas clouds. If we denote with $\mathfrak{M}_{max}$ the absolute magnitude of the nova at maximum, this relation is

$$\mathfrak{M}_{max} = -5 \log_{10} \bar{v} + \mathfrak{M}_1. \tag{10}$$

The constant $\mathfrak{M}_1$ may assume somewhat different values for different novae inasmuch as it contains a term log R_0, where R_0 is the radius of the original star. I have shown[5] that the relation (10) satisfactorily represents the data which are available at the present for ordinary novae. Extrapolating (10) to supernovae, it follows that for these stars

$$10^4\ km/\text{sec} < \bar{v} < 10^5\ km/\text{sec}. \tag{11}$$

From all of these considerations it becomes therefore highly probable that for an average supernova

$$\epsilon_k \gtrsim 1.5 \times 10^{-3}mc^2. \tag{12}$$

This means, for instance, that the kinetic energy of an iron atom ejected from a supernova presumably lies in the range

$$10^8 \text{ el. volts} < \epsilon_k(Fe) < 10^9 \text{ el. v} \tag{13}$$

Intergalactic and interstellar space are hence, through supernovae, continually supplied with *atomic rays* which are made up of high speed atoms and ions, the kinetic energy of which is proportional to their atomic weight. Some of the phenomena which atomic rays may cause on their journey through various stellar systems I have discussed previously.[6]

In the search for an interpretation of the spectra of supernovae, it will be well to keep in mind that velocities of the order contemplated in the preceding considerations will tend to displace and to widen absorption lines and emission lines to a degree not so far encountered in the spectra of any of the familiar celestial objects.

C. Cosmic Rays Generated during the Breakdown of the Electric Fields Which Are Built up through the Violent Fission of a Star.—Several processes which presumably take place in a supernova outburst would seem to be capable of producing cosmic rays. Some of these processes depend strongly on the radiation density and the relative concentrations of various stages of ionization of the ejected gas atoms. Since we do not have at our disposal sufficient data on the required physical characteristics of a supernova to discuss these modes of generation of cosmic rays in detail, we shall describe them in the second part of this paper in a general fashion only.

There is, however, imaginable one process capable of generating cosmic rays, the existence of which in supernovae appears extremely probable on the basis of available data on supernovae. This process is related to the creation of large differences in electric potential because of *random* separation of elementary particles of positive and negative charge.[8] If we disregard the systematic segregation of positive and negative ions, which is to be discussed in the second part of this paper, electric potential differences of the order[8]

$$\Phi \geqslant 10^9 \text{ volts} \tag{14}$$

can be built up through the violent random segregation of positive and negative charges during the fission in a supernova. Since I have discussed this process in another place[8] we proceed to consider briefly those processes which tend to systematically segregate electric charges of different signs.

D. Generation of Large Differences in Electric Potential Because of the Selective Action of the Light Pressure on Various Particles.—The kinetic energies, which atoms and ions, electrons and protons acquire in a directed flow of radiation depend on the character of the particle considered, as well as on the spectral distribution of this radiation. The ultimate value of the kinetic energy is essentially determined by the following processes.

(1) As long as the density of the ejected gas clouds is large enough to suppress any selective acquisition of energy, the final velocity $\bar{v}$ of all

particles will be the same. This case of the atomic rays was discussed in Section *B*.

(2) When the density, because of the expansion of the gas clouds, becomes so low that collisions do not any more seriously interfere with the accelerating action of the radiation, selective acceleration will take place. For instance, electrons will be accelerated more than protons; and atoms and ions, through the photoelectric effect and particularly through the efficient action of light pressure in strong resonance absorption lines, may acquire considerable kinetic energies. The problems involved here are of great interest, but cannot as yet be treated in detail in the case of supernovae because of lack of knowledge regarding the determining physical conditions in the neighborhood of a supernova.

(3) In both of the stages (1) and (2) of the expanding gas clouds, the selective action of the light pressure tends to segregate positive and negative charges. This segregation will result in the systematic building up of differences in electric potentials. We have no detailed knowledge regarding the total amount of the charges carried away, nor do we know what the ultimate distances of separation of these charges are. We know, however, that the light pressure in supernovae is sufficient to produce charges and separations such that the total potential energy $\mathfrak{E}_p$ due to these charges is of the order of the total energy $\mathfrak{E}_r$ emitted in the form of radiation, where $\mathfrak{E}_r$ is equal or greater than the energy $\mathfrak{E}_{vis} \cong 10^{48}$ ergs[7] carried away by the radiation in the visible spectrum. This permits us to estimate the possible limits of the differences in electric potential which may be built up temporarily because of the selective action of the radiation in a supernova outburst.

Suppose that the segregating action of the light pressure has created a space charge σ e. s. u./cm.^{3} in a volume $V = L^3$ surrounding the stellar remnant of a supernova. The total electric potential energy then is

$$\mathfrak{E}_p = \overline{E^2}L^3/8\pi = \alpha Mc^2 \tag{15}$$

where $\overline{E^2}$ is the averaged square of the field strength and α may be of the order $\alpha = 10^{-4}$. Therefore,

$$(\overline{E^2})^{1/2} = (8\pi\alpha Mc^2/L^3)^{1/2}. \tag{16}$$

We obtain the maximum potential difference Φ_{max} if the space charge is essentially of one sign, say negative on the stellar remnant and positive on the ejected gas clouds. We may then have

$$\Phi_{max} \cong L(\overline{E^2})^{1/2} = (8\pi\alpha Mc^2/L)^{1/2}. \tag{17}$$

If we put $M = \mu M_\odot$ and $L = Dr_\odot$ we obtain

$$\Phi_{max} \cong 8 \times 10^{19}(\mu/D)^{1/2} \text{ e. s. u.}, \tag{18}$$

with a radial field component of the average order

$$\overline{E} \cong \Phi_{\max}/Dr_{\odot} \tag{19}$$

and a total space charge Σ of the order

$$\Sigma = \overline{E}L^2 \cong (8\pi\alpha Mc^2L)^{1/2}. \tag{20}$$

The value of D, about one hundred days after maximum, according to the considerations of section *B* may be of the order $D = 10^6$ which, with $\mu = 1$ would result in

$$\Phi_{\max} \cong 8 \times 10^{16} \text{ e. s. u.} \tag{21}$$

and

$$\begin{aligned} \overline{E} &\cong 300 \text{ volts/cm.} \\ \Sigma &\cong 5 \times 10^{33} \text{ e. s. u.} \end{aligned} \tag{22}$$

E. Breakdown of the Electric Fields Surrounding the Stellar Remnant.—The space charges and the electric fields in the ejected gaseous masses will break down when the radiation from the stellar remnant decreases to a point where it is not any more able to support these space charges. At that stage the mean free paths of the individual corpuscles will also have become very large because of the exceedingly rapidly decreasing density of the expanding gas clouds. Breakdown of the enormous electric potentials will cause the production of elementary corpuscles whose kinetic energies are well *within* and *above* the range of those observed in cosmic rays. Supernovae thus may well be regarded as a powerful source of cosmic rays.

Baade and I have shown previously[1] that on the picture just discussed, combined with results, now available, of the frequency of supernovae, it is also possible to account for the total intensity of the cosmic radiation observed on the earth.

Finally, attention should be drawn to the fact that particles of different charge and mass will emerge with different kinetic energies from the collapse of the electric fields. In the first place, the energy of particles of equal sign will be the largest for the lightest particles. In the second place the kinetic energy will be larger for the particles which are located in initially large electric fields. The operation of these two effects may lead to a satisfactory understanding of the asymmetry in cosmic rays which causes the east-west effect.

The considerations sketched in this paper will be discussed in more detail in another place.

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[2] F. Zwicky, *Publ. Astr. Soc. Pacific,* **50,** 215 (1938); and **51,** 36 (1939).

[3] R. Minkowski, *Astrophys. Jour.*, **89**, 156 (1939).
[4] F. Zwicky, *Phys. Rev.*, **55**, 726 (1939).
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[6] F. Zwicky, *Ibid.*, **22**, 266 (1936).
[7] W. Baade and F. Zwicky, *Astrophys. Jour.*, **88**, 411 (1938).
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AN EXPERIMENT IN HUMAN VITAMIN A-DEFICIENCY

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The first observable symptom of vitamin A-deficiency in man and other mammals is a rise of visual threshold known as night-blindness. This response is based at least in part on the direct participation of vitamin A in a retinal cycle with the photosensitive pigment of the rods, rhodopsin.[2] With other conditions held constant, the sensitivity of the rods to light depends upon their rhodopsin content. As the level of vitamin A falls on a deficient diet, the concentrations of all components of the retinal cycle, including rhodopsin, decrease and the visual threshold reciprocally rises. Measurements of the rise and reversal of night-blindness therefore yield important information concerning the utilization of vitamin A *in vivo*. One set of such measurements upon a human subject has already been reported from this laboratory.[3] The present paper describes the results of a second experiment in which identical observational methods were employed.

The subject D. S. is 22 years of age, 5 feet and $10^1/_2$ inches in height and weighs 155 pounds. Throughout the course of the research he remained in excellent physical condition, ate very well and exercised regularly.

A standardized procedure was employed in all the measurements. Following 4 minutes' exposure to an intense light-adapting screen (4200 millilamberts), the absolute threshold of vision was measured periodically on alternate eyes during about 40 minutes in darkness. The test field for the threshold determinations is circular and subtends a visual angle of 2.1°. It is fixated 11.7° above the fovea, and is exposed by the subject for flashes of approximately $^1/_{50}$ second.

The experiment was begun with a period of vitamin A saturation, lasting 18 days, during which the normal diet was supplemented with 51,000 International Units of vitamin A daily, or from 10 to 25 times the accepted maintenance level.[4] Dark adaptation measurements during this period are shown as closed circles in figure 1. They are of characteristic form. The threshold falls rapidly to a first plateau, maintained until about the 11th

minute, then falls again to a second plateau, maintained thereafter. It has long been appreciated that the first segment is due to the dark adaptation of the cones, the second to that of the rods. The first plateau therefore represents the threshold of the completely dark adapted cones, the second that of the rods. The plateau intensities afford convenient indices for describing the course of the experiment. They are plotted separately in figure 2.

Following the saturation period the subject abruptly eliminated all dairy products, colored vegetables, liver, kidney and other selected foods from the diet, reducing its vitamin A content to 100–200 units per day.[4] Simultaneously the diet was supplemented with optimal amounts of vitamins B_1, B_2,

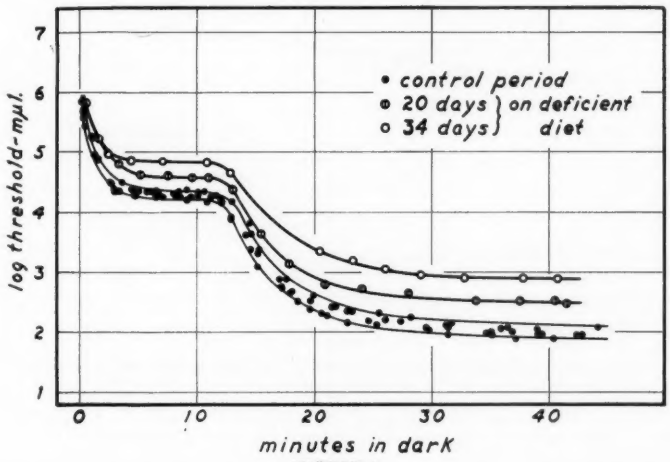

FIGURE 1

Dark adaptation during a period of vitamin A saturation (closed circles; 7 experiments), and at various intervals during vitamin A-deficiency (open circles). Each point represents a single determination of the absolute threshold of the right eye to white light. Threshold intensities in millimicrolamberts = 10^{-6} millilamberts.

C, D, calcium phosphate and ferrous sulphate.[5] Under these circumstances the diet probably is deficient in vitamin A alone.

On this diet the thresholds of both cones and rods rose regularly. The effects on the form of the dark adaptation function are shown in figure 1, and the changes in the plateaus in figure 2. The rise of log threshold is greater in the rods than in the cones, and greater in the plateaus than in the descending portions of either segment. The plateaus remain inside their normal ranges for the first 3 or 4 days on the deficient diet. Thereafter the log thresholds rise linearly in both cones and rods. It is an important fact that the lines which best fit the complete data for both rods and cones originate

on the first day of the deficiency. Apparently, following even a period of vitamin A saturation, the ascent into night-blindness on a deficient diet begins at once.

On the 34th day of the deficiency the cone plateau had risen 3.4 times, the rod plateau 9.1 times above their average normal levels. This mild state of night-blindness was completely reversed with a single administration of carotene. The course of the reversal experiment is shown in figure 3. A preliminary standard light and dark adaptation showed the subject

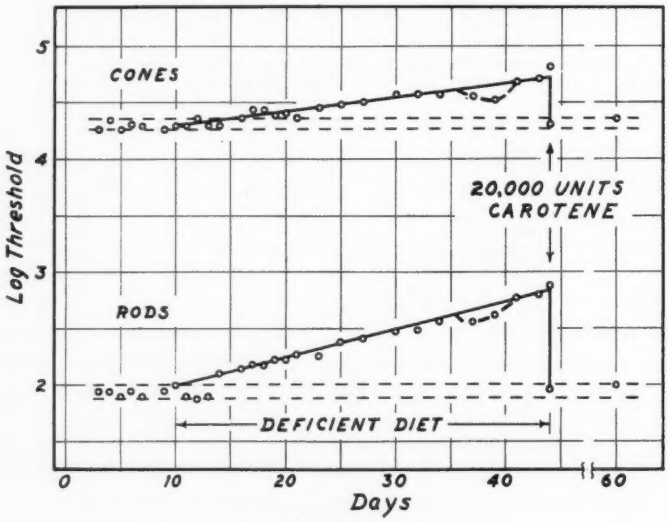

FIGURE 2

Thresholds of the completely dark adapted cones and rods (cone and rod "plateaus"), during 34 days on a vitamin A-low diet, and during previous and subsequent normal periods. The normal ranges of variation for cones and rods are enclosed within broken lines. The depression of thresholds on the 35th to 41st days of the experiment is due to a slight break in the diet on the 35th day. On the 34th day of the deficiency the night-blindness was reversed with carotene; the details of this experiment are shown in figure 3.

to be night-blind. Had he been normal the cone plateau would have lain within the upper pair of broken lines in figure 3, the rod plateau within the lower pair. After dark adaptation was complete, the subject was given 2 gelatine capsules of carotene in cottonseed oil (Smaco; about 20,000 units) orally with water. For 12 to 14 minutes the threshold remained constant. This is the "latent period" noted previously,[3] and presumably associated with the dissolution of the capsules and passage of the oil to the gut. Then the rod threshold fell rapidly, entered the normal range within about 90

minutes after the oil had been taken and became stable within about 100 minutes. Immediate repetition of the standard light and dark adaptation procedure showed both cone and rod plateaus to have entered their normal ranges.

The results of this research differ from our previous experiment[3] only in time relations. The rise in log threshold per day of the deficiency—the slopes of the lines in figure 2—is in the present experiment 0.012 in the cones and 0.025 in the rods. Corresponding values for the previous subject had been about twice as great: 0.022 in the cones and 0.054 in the rods. In the present instance the "latent period" following ingestion of carotene is about half as long, and the subsequent descent of the night-blind threshold to normal about twice as rapid as in the previous subject.

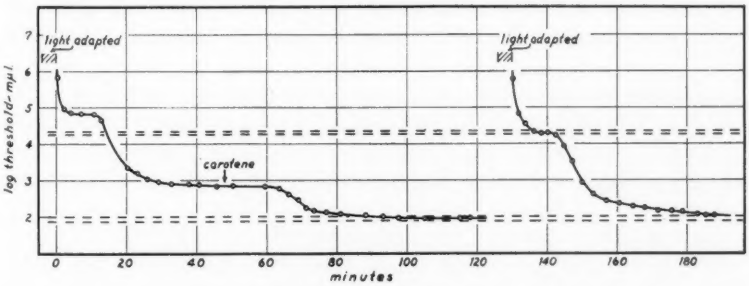

FIGURE 3

Reversal of night-blindness with carotene. Following a standard light adaptation, the measurement of dark adaptation shows both cone and rod plateaus to lie above their normal ranges (shown by broken lines). After dark adaptation is complete, 20,000 units of carotene are administered in gelatine capsules orally. For a "latent period" of 12–14 minutes the rod threshold remains constant. Then it falls rapidly to normal. Immediate repetition of the standard adaptation procedure shows both cone and rod plateaus to have entered their normal ranges.

The speed of rise of night-blindness in this type of experiment may vary much more widely than these two researches indicate. Our more recent work has shown that on an identical régime and within comparable periods about half of our subjects develop no night-blindness at all. The basis for this sharp variation in response is still unknown. It seems to have little to do with the vitamin A reserve since all our subjects have been "saturated" with vitamin A before beginning the deficient diet.

The parallelism in response of cones and rods to vitamin A-deficiency has been noted previously.[6, 3, 7] One obvious inference to be drawn from it is that vitamin A is the precursor of cone photopigments in addition to rhodopsin. But this is not the only plausible possibility, and in default of direct chemical information the significance of the cone changes remains uncertain.

The linearity of rise of log threshold during the deficiency appears to possess the following significance. Hecht[8] has shown that the photodecomposition of rhodopsin is first-order, and is governed by an equation of the form, $-dr/dt = kIr$, in which k is a constant, and r, t and I are respectively the rhodopsin concentration, time and light intensity. If it be assumed that at the threshold the flash of constant duration, dt, decomposes a constant quantity of rhodopsin, dr, the expression reduces to $Ir = constant$. That is, the threshold intensity is inversely proportional to rhodopsin concentration. A linear rise of log threshold therefore corresponds to a simple logarithmic decrease in the concentration of rhodopsin, and this in turn to a logarithmic decrease in the available vitamin A, with which rhodopsin is in retinal equilibrium. This is almost the simplest result that could have been anticipated. It means that each day of the deficient diet a constant fraction of what rhodopsin and vitamin A remain is lost.

In human vitamin A-deficiency experiments recently reported by Hecht and Mandelbaum,[7] though the rise of night-blindness was much as we have described it, its reversal on vitamin A administration occupied about two months. This is in striking disagreement, not only with our own results, but with clinical reports that severe night-blindness is reversed within periods of 24 to 48 hours.[9] The basic deficiency diets which all of us have employed are almost certainly low in other dietary essentials than vitamin A. Hecht and Mandelbaum's procedure differed significantly from ours in omitting supplements of these factors. It may be that in this case conditions other than simple lack of vitamin A contribute to the rise of night-blindness, and produce effects which are only slowly reversed by vitamin A administration and the return to a normal diet.

[1] Henry Fellow.

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[5] Daily supplements were taken of about 280 International Units of vitamin B_1 and 160 Sherman Units of B_2 in brewer's yeast tablets, 50 mgm. ascorbic acid, about 1700 International Units of vitamin D, 2 grams dicalcium phosphate and 9 grains ferrous sulphate. We are much indebted to the Abbott Laboratories of North Chicago, Ill., for supplying these materials and the halibut liver oil used as the source of vitamin A.

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[9] Aykroyd, W. R., *Lancet*, **1**, 824 (1930); Mori, personal communication, cited in the Medical Research Council Report on Vitamins (1932). Note also the report of partial reversal of a mild experimental night-blindness within 2 hours by Jeghers (*Jour. Am. Med. Assn.*, **109**, 756 (1937)). Edmund and Clemmesen (*Acta Med. Scand.*, **89**,

69 (1936)) have reported complete cures of night-blindness within 7 to 10 minutes following intramuscular injection of vitamin A concentrates; but this work requires confirmation with more accurate procedures (cf. Groth-Petersen, E., *Acta Med. Scand.*, **95**, 110 (1938)).

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*THE STEADY POTENTIAL OF THE HUMAN EYE IN SUBJECTS WITH UNILATERAL ENUCLEATION**

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In some previous communications[1, 2] a method was described for the direct study of the polarity potential in the human eye *in situ*, and the reliability of the measurements, typical results and individual differences were reported. The subjects in these former studies were all normal people in the respect that each had a pair of functioning eyes free from obvious pathology and exhibiting conjugate motility. By different electrode leads the eyes were measured separately and in combination when rotated laterally 30° to either side of the primary line of regard; and the relationships between the potentials found for these different leads were examined. Since each eyeball appears to constitute a separate and independent source of biological potential and the distance between their centers is ordinarily about 62 mm., it seems probable that the area of electrification that extends from each eye partially overlaps that of the other. The potential of one eye therefore cannot be measured independent of some influence from its mate. The desirability of investigating eye potentials in some people who each possess only one active eye naturally suggested itself.

The present study concerns data secured on a group of subjects each of whom has suffered unilateral enucleation and in consequence wears one artificial eye. Eye potential measurements were taken in the standard manner on a total of thirteen people with enucleation who coöperated in this investigation; but because of complicating pathologic conditions in the active eye the records for four cases could not be used in the final comparison. Results are therefore limited to five cases of right-eye and four cases of left-eye enucleation, a total of nine cases.

All subjects, whether belonging to the enucleation group or to the normal comparison group, fulfilled the same measurement routine, at least so far as they were able. This routine included certain physiological tests such as pulse rate, blood pressure and several visual tests as well as some physical measurements and queries about health and physiological state. Four small metal foil electrodes (as illustrated in a previous communica-

tion[2]) were placed about each eye, the artificial eye as well as the active one. None of the subjects complained or gave evidence of any discomfiture from these electrodes. As might be expected, all of the enucleates were at first a little fearful of the examination. Especially was this true of the two youngest, Nos. 62 and 61, and this condition militated against securing as much data for them as would have been desirable.

The results previously reported,[1, 2] whether for individuals or as group means, have in all instances represented averages for 30° rotations (lead-off angles) to the right and to the left inclusive. This method of exhibiting the measured potential has masked a certain well defined tendency in the data which will now be considered. As a standard against which to compare the enucleation groups data for fifteen normal subjects are presented in table 1.** The leads and the electrode placements about the eyes are indicated in the heading of the table. All of the values expressed in millivolts in the table, with the exception of those in the two columns under Lead 4, are of plus sign; the values under Lead 4 are of minus sign. The means for Leads 1 and 2, respectively, show a rather clear tendency toward a difference in measured potential according as the eye is turned 30° right or 30° left. When a given eye is turned temporalward, the registered potential tends to be appreciably higher than when it is turned nasalward. This difference may arise from the fact that the two lateral electrodes for each eye cannot occupy positions in the same coronal plane. The nasal electrode is anterior to the temporal electrode by about 17 mm. and is about 6 mm. farther from the center of the bulbus. The temporal electrode is about 12 mm. posterior to the apex of the cornea and therefore relatively near to the meridian of the eyball while the nasal electrode is 4 mm. anterior to the plane of the cornea. Furthermore the two nasal electrodes are separated by a distance scarcely greater than 24 mm. and therefor lead from areas of electrification which though of opposite sign are so near as definitely to influence each other. These considerations do not wholly dominate the measured phenomena since there are some exceptions to the rule: Nos. 2, 80 and 123 are exceptions in the case of both eyes, while Nos. 1 and 111 deviate from the rule with one eye each. At present these discrepancies cannot be explained.

The difference for right and left seems negligible when the two electrodes are on the temples (Lead 3) and also when the two nasal electrodes are used (Lead 4). No difference of the character mentioned appears when the two eyes are connected in parallel for registration as in Lead 5. When the potential is measured by means of the electrodes placed above and below the eye it appears that the two directions of movement, each covering visual angles of 30°, usually give potentials that do differ. The averages are larger when the eye is directed upward than when directed downward. Deviations from this tendency occur particularly in younger subjects.† It

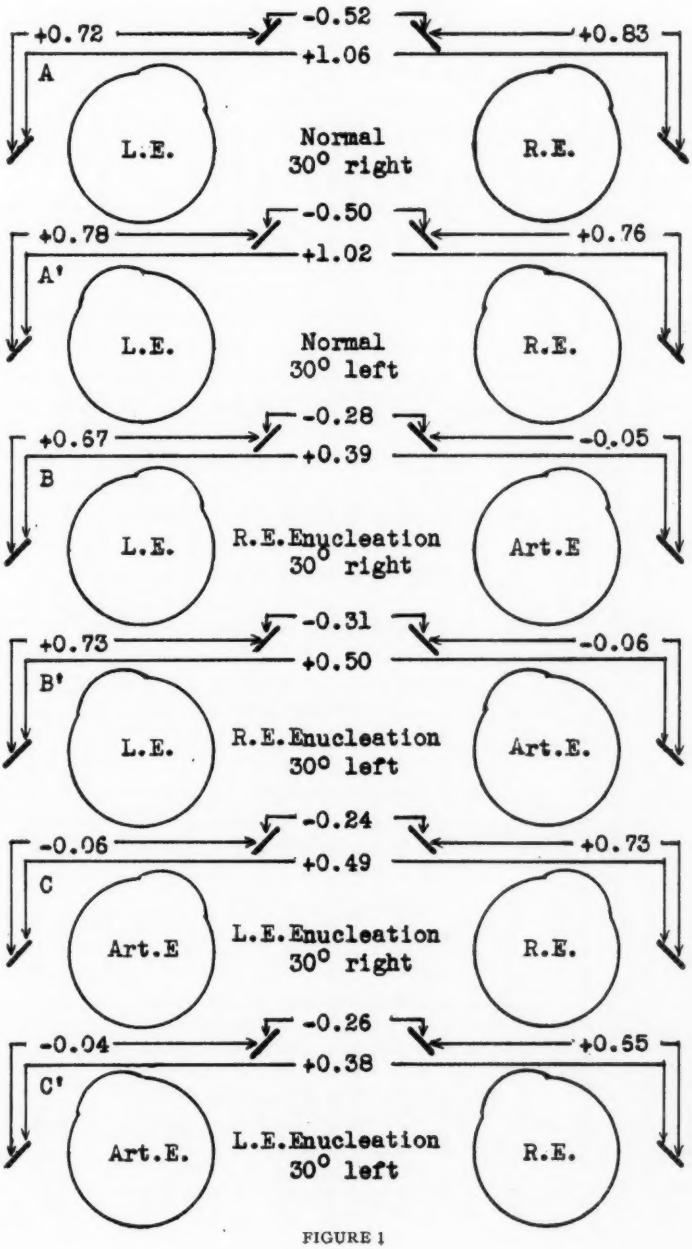
+0.72
-0.52
+0.83
+1.06
A
L.E.
Normal
30° right
R.E.
+0.78
-0.50
+0.76
+1.02
A'
L.E.
Normal
30° left
R.E.
+0.67
-0.28
-0.05
+0.39
B
L.E.
R.E.Enucleation
30° right
Art.E
+0.73
-0.31
-0.06
+0.50
B'
L.E.
R.E.Enucleation
30° left
Art.E.
-0.06
-0.24
+0.73
+0.49
C
Art.E
L.E.Enucleation
30° right
R.E.
-0.04
-0.26
+0.55
+0.38
C'
Art.E.
L.E.Enucleation
30° left
R.E.

FIGURE 1

TABLE 1

OCULAR POLARITY-POTENTIALS IN 15 NORMAL SUBJECTS

Values in millivolts for lead-off angles of 30° left, right, above and below the primary line of regard. (Values under Lead 4 are minus; all other leads are plus.)

CASE NO.	AGE	SEX	HAND DOM.	EYE DOM.	VIS. ACU.	LD. 1 −LE− L	LD. 1 −LE− R	LD. 2 −RE− L	LD. 2 −RE− R	LD. 3 −LE RE− L	LD. 3 −LE RE− R	LD. 4 LE− −RE L	LD. 4 LE− −RE R	LD. 5 −LE− −RE− L	LD. 5 −LE− −RE− R	LD. 6 L−E U	LD. 6 L−E D	LD. 7 $\overline{RE}$ U	LD. 7 $\overline{RE}$ D	LD. 8 $\frac{\overline{LE}}{U}$	LD. 8 $\frac{\overline{RE}}{D}$
100	8	F	R	L	100	0.97	0.80	0.72	0.97	1.22	1.22	0.54	0.49	1.00	1.00	0.81	0.75	0.52	0.79	0.72	0.70
32	10	F	R	L	100	0.73	0.62	0.72	0.76	0.73	0.86	0.54	0.43	0.67	0.64	0.62	0.57	0.58	0.63	0.47	0.45
52	12	M	L	L	100	0.67	0.61	0.55	0.72	1.00	0.92	0.36	0.47	0.71	0.76	0.94	0.50	0.64	0.32	1.00	0.50
108	12	M	R	R	100	0.96	0.77	0.85	1.04	1.43	1.35	0.51	0.49	0.86	0.86	0.60	0.78	0.63	0.86	0.56	0.76
98	12	F	R	L	100	0.51	0.31	0.62	0.72	0.86	0.55	0.45	0.48	0.60	0.60	0.69	0.66	0.54	0.43	0.70	0.63
45	12	M	R	L	100	0.77	0.74	0.77	0.83	1.17	1.20	0.46	0.46	0.57	0.52	0.28	0.47	0.33	0.39		
111	14	M	R	L	100	0.62	0.51	0.49	0.45	0.80	0.89	0.31	0.33	0.65	0.59	0.40	0.34	1.29	0.43	1.06	0.38
80	18	M	R	L	105	0.87	0.87	0.75	0.51	0.95	0.81	0.57	0.54	0.65	0.60	0.98	0.62	0.88	0.60	1.03	0.64
123	20	M	R	R	100	0.57	0.56	0.81	0.81	0.91	0.91	0.39	0.43	0.66	0.66	0.57	0.30	0.38	0.36	0.43	0.29
2	21	F	R	L	100	0.89	1.06	1.16	1.09	0.86	1.08	0.89	0.92	0.89	0.84	0.77	0.62	1.59	1.00	0.72	0.72
1	21	M	R	R	105	0.65	0.70	0.55	0.67	0.92	0.97	0.36	0.31	0.78	0.81	0.64	0.31	0.66	0.37	0.67	0.36
26	21	M	R	R	105	0.95	0.89	0.97	1.17	1.44	1.56	0.63	0.74	1.03	1.14	1.53	0.39	0.50	0.39	1.08	0.28
130	22	M	R	R	100	0.72	0.70	0.72	0.80	1.08	1.08	0.46	0.46	0.64	0.69	0.82	0.53	0.79	0.48	0.77	0.51
28	23	M	R	R	105	0.90	0.97	0.75	0.95	1.00	1.06	0.49	0.79	0.69	0.77	0.74	0.71	**	**	0.95	0.77
54	25	F	R	L	100	0.95	0.75	0.92	1.00	1.00	1.44	0.53	0.48	1.11	1.09	0.81	0.36	1.22	0.42	1.00	0.56
					Mn	0 78	0.72	0.76	0.83	1.02	1.06	0.50	0.52	0.77	0.77	0.75	0.53	0.75	0.53	0.80	0.54
					S. D.	0.15	0.18	0.17	0.20	0.20	0.25	0.13	0.16	0.16	0.18	0.27	0.16	0.36	0.20	0.25	0.16
					P. E.$_m$	0.03	0.03	0.03	0.04	0.04	0.04	0.02	0.03	0.03	0.03	0.05	0.03	0.07	0.04	0.05	0.03

** Indefinite because of winks, poor fixation, etc.

TABLE 2

OCULAR POLARITY-POTENTIALS IN 9 SUBJECTS WITH UNILATERAL ENUCLEATION†

Values in millivolts for lead-off angles of 30° left, right, above and below the primary line of regard. (Values for Lead 4 are minus, also for the artificial eye under Leads 1 and 2. Other values in table are plus.)

CASE NO.	AGE	SEX	HAND DOM.	ENUCL. EYE	YRS., MOS. SINCE ENUCL.	VIS. ACU.	LD. 1 −LE− L	LD. 1 −LE− R	LD. 2 −RE− L	LD. 2 −RE− R	LD. 3 −LE RE− L	LD. 3 −LE RE− R	LD. 4 LE− −RE L	LD. 4 LE− −RE R	LD. 5 −LE− −RE− L	LD. 5 −LE− −RE− R	LD. 6 LE U	LD. 6 LE D	LD. 7 RE U	LD. 7 RE D	LD. 8 LE U	LD. 8 RE D
62	9	M	R	R	1–10	105	0.97	0.76	0.08	0.05	0.63	0.55	0.34	0.30			0.74	0.80	0.21	0.18		
							0.89	0.76	0.05	0.08	0.65	0.51					0.80	0.82				
41	12	M	R	R	2–6	105	0.83	0.67	0.06	0.04	0.66	0.46	0.24	0.24	0.49	0.37	0.62	0.49	0.24	0.19	0.43	0.27
							0.74	0.60	0.03	0.07	0.54	0.37										
43	26	F	R	R	8–0		0.84	0.84	0.09	0.09	0.47	0.36	0.36	0.36	0.43	0.38	1.19	0.67	0.17	0.22	0.73	0.43
							0.80	0.77	0.08	0.09	0.44	0.36										
44	47	F	R	R	18–0	100	0.79	0.79	*	*	0.54	0.45	0.31	0.29	0.45	0.42	0.44	0.85	0.09	0.12	0.36	0.47
46	64	M	R	R	0–9	80	0.35	0.46	0.09	**	0.27	0.22	**	0.22	0.17	0.11	0.35	0.19	0.14	0.11	**	**
							0.35	0.42			0.26	0.21										
						Ave.	0.73	0.67	0.06	0.05	0.50	0.39	0.31	0.28	0.38	0.32	0.69	0.64	0.17	0.16	0.51	0.29
61	10	M	R	L	8–0	110	0.13	0.08	0.76	0.76	**	**	0.21	0.21					0.62	0.39		
									0.74	0.76	0.53	0.66										
77	21	M	R	L	4–0	110	*	*	0.92	0.92	0.45	0.57	0.27	0.27	0.35	0.55	*	*	0.80	0.62	0.29	0.29
									0.70	0.80	0.45	0.63										
83	31	M	R	L	1–3	80	*	0.18	0.41	0.53	0.29	0.32	0.18	0.20	0.18	0.20	*	*	0.33	0.28	0.16	0.12
							*	*	0.45	0.55	0.30	0.39							0.42	0.51		
23	71	F	R	L	2–5	110	*	*	0.60	0.82	0.31	0.41	0.36	0.28	0.27	0.38	0.11	0.11	0.87	1.12	0.27	0.19
							0.11	0.10			0.31	0.42										
						Ave.	0.04	0.06	0.65	0.73	0.38	0.49	0.26	0.24	0.27	0.38	0.04	0.04	0.61	0.58	0.24	0.20

† Four other cases of unilateral enucleation were examined and records were taken, but because of complicating conditions such as blindness in the active eye they have not been included in the present comparison.

* No measurable deflection; counted zero potential.

** Indefinite because of winks, poor fixation, etc.

seems obvious, moreover, that individuals vary more widely in the conformation of the tissues above and below the eye where electrodes may be located than in those for lateral locations. This fact of individual anatomical difference with age operates against the comparability of eye potentials taken from vertical leads and in favor of those from lateral leads as the more standard measurement.

With normal data before us we may now turn to the consideration of results gotten on the nine subjects with unilateral enucleation. Their results are presented in table 2, tabulated as far as possible in the same arrangement as has been used for the normal group. The five right-eye enucleates are listed at the top of the table and have been averaged separately as have the four left-eye enucleates in the lower part of the table. For Leads 1, 2 and 3, but rarely in other leads, a second test was usually made. This does not mean a second visit of the subject to the laboratory, but in each instance the retest was in the same experimental session but separated from the first test by fifteen minutes or more. The striking thing about the retests (second horizontal line of values for each subject) is that they are as a rule lower than the first tests. This is a further demonstration of a phenomenon previously mentioned,[2] that is, an elevation of the potential through the psychological factor of apprehension associated with the measurement situation. When the subjects found that they experienced no hurt or discomfort and that it was not difficult to do what was asked of them, they felt more at ease in the situation and concomitantly with this it is seen that the potential was lower. In one or two instances this was not the case, as in No. 83, Leads 2, 3 and 7.

The most striking thing in table 2 is the great discrepancy between the millivolt values as shown for the active eye and those for the artificial eye as represented in Leads 1 and 2. The active eye for each group of enucleates demonstrates bioelectrical values for right and left rotation which correspond substantially with results found on the normal subjects. As a rule the laterality differential is also found for the enucleates: the active eye, when looking temporalward, demonstrates a higher potential than when looking nasalward.

Measurements on Lead 3, the bitemporal electrode placement, differ widely between the enucleates and the normals. In the latter there is practically no difference between left and right movements, but with the enucleates, since one temporal electrode is approximately 70 mm. from the active eye, there is a distinct difference according to which way this eye turns. When the left eye (active) is rotated temporalward, the average is +0.50, which is half the value found for the normals; but when the rotation is nasalward, the average is only +0.39 mv. The complement of these results is found for the right eye (active).

The nasal electrodes for the enucleates are both on the same side of the

active eye. They show less laterality differential for the two directions of movement; and the average potential falls definitely below the value secured on normal two-eyed people, but is not reduced proportionately so much as in the case of the bitemporal leads. The parallel arrangement, Lead 5, which for the normals gave an average of the values found for the two eyes measured separately, in the enucleates gave a result close to one-half that found for the single active eye measured separately.

TABLE 3

SUMMARY OF COMBINED DATA ON OCULAR POLARITY-POTENTIALS FOR 30° ROTATIONS FOR 5 CASES OF RIGHT-EYE AND 4 CASES OF LEFT-EYE ENUCLEATION

(Values in millivolts)

ELECTRODE PLACEMENT	ACROSS ACTIVE EYE		ACROSS ARTIFICIAL EYE	
FIXATION DIRECTION	TEMPORALWARD	NASALWARD	TEMPORALWARD	NASALWARD
N (observations)	16	16	13	14
Mn	+0.73	+0.67	−0.05	−0.02
S. D.	0.18	0.15	0.05	0.07
P. E.$_m$	0.032	0.027	0.009	0.013
ELECTRODE PLACEMENT FIXATION DIRECTION OF ACTIVE EYE	BITEMPORAL (LD. 3)		BINASAL (LD. 4)	
	TEMPORALWARD	NASALWARD	TEMPORALWARD	NASALWARD
N (observations)	16	16	8	9
Mn	+0.49	+0.38	−0.28	−0.27
S. D.	0.13	0.10	0.05	0.06
P. E.$_m$	0.023	0.018	0.013	0.014
ELECTRODE PLACEMENT FIXATION DIRECTION OF ACTIVE EYE	LATERAL PARALLEL CONNECTION OF BOTH EYES (LD. 5)		ABOVE AND BELOW ACTIVE EYE	
	TEMPORALWARD	NASALWARD	UP	DOWN
N (observations)	7	7	11	11
Mn	+0.38	+0.30	+0.65	+0.61
S. D.	0.13	0.11	0.25	0.26
P. E.$_m$	0.036	0.030	0.054	0.056
ELECTRODE PLACEMENT FIXATION DIRECTION OF ACTIVE EYE	ABOVE AND BELOW ARTIFICIAL EYE		VERTICAL PARALLEL CONNECTION OF BOTH EYES (LD. 8)	
	UP	DOWN	UP	DOWN
N (observations)	8	8	6	6
Mn	+0.12	+0.12	+0.37	+0.30
S. D.	0.08	0.08	0.18	0.12
P. E.$_m$	0.019	0.019	0.054	0.036

Measurements for vertical rotations of the single active eye in enucleates show results which correspond fairly well with those obtained on the comparison group. Both groups demonstrate larger potentials when the eye is rotated upward than when rotated downward, and the results for 30° of upward rotation correspond fairly well with those for 30° of lateral rotation. The series arrangement with vertical rotation, Lead 8, for the nor-

mals gave what amounts to a mean value for the two eyes, but in the enucleates approached half the value obtained for the single active eye.

A combined summary of the data procured on the two groups of enucleates is given in table 3. The averaged results found for the active eye are set in comparison with those obtained from similar regions around the artificial eye, and in each instance rotations have been classified as temporalward or nasalward, up or down. The signs of the potentials have been entered and statistical constants are given.

In figure 1 comparable sets of measurements for 30° lateral eye positions, taken from tables 1 and 2, are given on a standard diagram which is drawn to scale and in horizontal section represents the positions of the electrodes relative to the eyeballs. In A and A′, respectively, it will be noted that the bitemporal lead gives a value which corresponds closely to the sum of the single-eye potentials minus the binasal-lead potential. This relationship would be expected on theoretical grounds and has been previously considered.[1, 2]. The group of enucleates, B and B′ and C and C′, on the same grounds should show bitemporal-lead values which equal the value for the single active eye minus the binasal-lead potential and the potential found around the artificial eye (vacant orbit). Reference to the four diagrams mentioned will show that in each the expected result is substantially borne out although not quite so closely as in the averages for the normal comparison group.

The data gathered on the group of unilateral enucleates indicate that the region immediately surrounding the artificial eye is electrified from the active eye as source with polarization characteristics and values of potential according to the rotation direction of the active eye and approximately proportional to the distance from it. In some subjects no measurable potential was found around the artificial eye associated with lateral rotations of the active eye. In most instances a small potential, usually of reversed polarity, was demonstrable. With vertical rotations the electrified areas above and below the artificial eye have respectively the same signs and in most instances show potentials which amount to a fourth or more of the value found for the active eye. Nos. 77 and 83 are exceptional in showing no measurable potentials about the artificial eye associated with vertical rotation. Individual differences in the spread of electrification from the eyeballs to adjoining tissues appear to be quite marked.††

Conclusions.—1. Eye-potential measurements taken in the standard manner on nine people with unilateral enucleations, including both males and females of widely varying ages, demonstrated values for the single active eye which correspond closely with the comparable values found for normal two-eyed people.

2. The individual eye when turned 30° right or left from the primary line of regard registers an average potential of about 0.7 mv. When turned

temporalward the value tends to be higher than when the eye is turned nasalward. This lateral differential appears to be related to the necessarily asymmetrical placement of the electrodes on the two sides of the eye.

3. The enucleates agree with the normals in showing larger potentials when the eyes are directed upward than when directed downward by an equal amount. This difference, characteristic in adults, is reversed in children (girls 10–12 yrs.); therefore lateral deviations of the eyes are to be preferred as a standard comparable measure.

4. In most instances the region about the artificial eye of an enucleate shows quite small potentials which have the same sign as the potential registered from the active eye when it is deviated vertically, but which are of opposite sign when the active eye is turned laterally.

5. The bitemporal lead for the enucleates gives a result strikingly different from that for the two-eyed group: right and left deviations do not equal each other in potential and the registered value is much less than for the one active eye, whereas for normals Lead 3 exceeds Leads 1 or 2.

6. The normal relationship, Lead 1 plus Lead 2 minus Lead 4 equals Lead 3, in the results of the enucleates changes to: active-eye lead minus artificial-eye lead and Lead 4 equals Lead 3.

7. Individual differences in the spread of electrification to adjoining tissues from the active eye of enucleates or from each of a pair of eyes appear to be quite marked.

8. Repeated measurements within the same experimental session showed a tendency toward lower potentials in the enucleates. This is interpreted as being due in part to a decrease of fear and apprehension when the subjects found that the test was free from hurt or discomfort.

* The expenses of this investigation were met in part by a grant from the Knight Fund of Yale University School of Medicine. The writer wishes also to express his appreciation to Dr. Arthur M. Yudkin for assistance in securing the coöperation of subjects used in the study.

** No. 52 has been used here in place of No. 122 in table 1 of the earlier communication.[1]

† Data on 20 Girl Scouts 10 to 12 years of age confirm this generalization.

†† Further confirmation of this is found in other data on a group of 47 subjects normal and pathologic (all having two active eyes). In examining these subjects an extra pair of electrodes was placed on the forehead parallel to the temporal electrodes and 6 cm. above them. The electrodes on the forehead were therefore approximately as far away from the eyes as the eyes are separated from each other. Right and left lateral deviations of 30° were recorded in terms of the bitemporal lead and also from the forehead lead. On half the cases, 23, there was no measurable potential from the forehead electrodes. Of course this does not mean that potential was wholly absent. The other 24 subjects showed averages for the bitemporal and forehead leads, respectively, as follows:

Mn + 1.04, S. D. 0.29,.P. E_m 0.041; Mn + 0.14, S. D. 0.06, P. E_m 0.008.

[1] W. R. Miles, "The Steady Polarity Potential of the Human Eye," *Proc. Nat. Acad. Sci.*, **25**, 25–36 (1939).

[2] W. R. Miles, "Reliability of Measurements of the Steady Polarity Potential of the Eye," *Ibid.*, **25**, 128–136 (1939).

TEAM TESTS FOR GENERALS AND SPECIFICS

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The resolution of six tests into three generals and six specifics in two different ways[1] raises some interesting questions about the determinability of the generals and specifics in the two solutions and about the relations of the generals in the two solutions.[2] The team tests for either generals or specifics are unit vectors along the projections of those vectors upon the space of the tests and the squares of those projections give the variances of the known parts of the generals or specifics. The team tests for the specifics are the same in the two solutions,[3] but those for the generals are different. The projection of a vector upon a space may be obtained from the formula

$$\text{proj. of } f = (f \cdot f_1)f_1 + (f \cdot f_2)f_2 + f \cdot f_3)f_3 + \ldots \qquad (1)$$

where $f_1, f_2, f_3, \ldots$ are any orthogonal set of unit vectors in that space and such a set may be obtained directly in succession from the tests $x_1, x_2, x_3, \ldots$ by taking $f_1 = x_1, f_2 = b(x_2 - af_1), f_3 = e(x_3 - cf_2 - df_1), \ldots$, by expressing the conditions for orthogonality which will determine a, or c and d, etc., and by so determining $b, e, \ldots$ as to make successive f's unit vectors. The set of f's determined and used was[4]

$$\begin{aligned}
f_1 &= x_1, f_2 = -0.6759x_1 + 1.2070x_2,\\
f_3 &= -0.0715x_1 - 0.1644x_2 + 1.0224x_3,\\
f_4 &= -0.2139x_1 - 0.7629x_2 - 0.0570x_3 + 1.3546x_4,\\
f_5 &= 0.0656x_1 - 0.6779x_2 + 0.0326x_3 + 0.0601x_4 + 1.1668x_5,\\
f_6 &= -0.4336x_1 - 1.3708x_2 - 0.0888x_3 - 0.5206x_4 + 0.0317x_5 + 2.2777x_6.
\end{aligned}$$

Next, applying (1) to I, II, III and I′, II′, III′ as determined by their factor loadings, we find the projections

$$\begin{aligned}
\mathrm{I}_0 &= 0.8000f_1 + 0.3042f_2 + 0.0322f_3 + 0.0963f_4 - 0.0295f_5 + 0.1951f_6\\
&= 0.4850x_1 + 0.0410x_2 + 0.0091x_3 + 0.0270x_4 - 0.0282x_5 + 0.4444x_6\\
\mathrm{II}_0 &= 0f_1 + 0.7242f_2 + 0.0036f_3 + 0.0784f_4 + 0.2039f_5 + 0.1151f_6\\
&= -0.5431x_1 + 0.5177x_2 - 0.0044x_3 + 0.0586x_4 + 0.2416x_5 + 0.2621x_6\\
\mathrm{III}_0 &= 0f_1 + 0f_2 - 0.1022f_3 - 0.2652f_4 + 0.4514f_5 - 0.3298f_6\\
&= 0.2367x_1 + 0.3653x_2 - 0.0454x_3 - 0.1604x_4 + 0.5163x_5 - 0.7513x_6.
\end{aligned}$$

The variances are $I_0^2 = 0.7818$, $II_0^2 = 0.5855$, $III_0^2 = 0.3934$ and the team tests may be had by scaling up I_0, II_0, III_0 by dividing by the square roots of the variances. The coefficients of correlation between the pairs of team tests and the angles between them are

$$r_{I_0II_0} = 0.3612\ (68^0.8),\ r_{I_0III_0} = -0.1920(101^0.1),\ r_{II_0III_0} = 0.0686(86^0.1).$$

It will be observed that the projections of the orthogonal vectors I, II, III are far from orthogonal.

For the second solution we have

$$\begin{aligned} I_0' &= 0.6524f_1 + 0.5951f_2 + 0.0629f_3 + 0.1883f_4 - 0.0577f_5 + 0.3817f_6 \\ &= 0.0361x_1 + 0.0801x_2 + 0.0178x_3 + 0.0529x_4 - 0.0552x_5 + 0.8695x_6 \\ II_0' &= 0f_1 + 0.4910f_2 - 0.0933f_3 - 0.2142f_4 + 0.2846f_5 - 0.4763f_6 \\ &= -0.0543x_1 + 1.2313x_2 - 0.0316x_3 - 0.0251x_4 + 0.3170x_5 - \\ &\quad 1.0848x_6 \\ III_0' &= 0f_1 + 0f_2 + 0.0527f_3 + 0.0329f_4 - 0.1752f_5 - 0.4413f_6 \\ &= 0.1690x_1 + 0.6899x_2 + 0.0855x_3 + 0.2638x_4 - 0.2184x_5 - \\ &\quad 1.0051x_6. \end{aligned}$$

The variances are $I_0'^2 = 0.9682$, $II_0'^2 = 0.6035$, $III_0'^2 = 0.2293$. The correlation coefficients and angles are

$$r_{I_0'II_0'} = 0.0625(86^0.4),\ r_{I_0'III_0'} = -0.3159(108^0.4),\ r_{II_0'III_0'} = 0.3988(66^0.5).$$

The angles again are not right angles.

In respect to the specifics we shall tabulate the coefficients of the x's for the unit team tests, and state the variances for the two solutions.

	x_1	x_2	x_3	x_4	x_5	x_6	VARIANCES FIRST	VARIANCES SECOND
s_1	1.3038	−0.0698	−0.0155	−0.0461	0.0481	−0.7574	0.6120	0.9765
s_2	−0.0434	2.0987	−0.0119	−0.1718	−0.3976	−1.4877	0.6607	0.4303
s_3	−0.0197	−0.0242	1.0284	−0.0283	0.0343	−0.1968	0.9941	0.9904
s_4	−0.0414	−0.2482	−0.0200	1.4525	0.0369	−0.8164	0.9283	0.9558
s_5	0.0538	−0.7149	0.0302	0.0459	1.1672	0.0619	0.6812	0.8356
s_6	−0.4336	−1.3708	−0.0888	−0.5206	0.0317	2.2777	0.3632	0.0104
							4.2394	4.1990
The sum of the variances of the known parts of the generals							1.7606	1.8010
							6.0000	6.0000

We have therefore two complete variance analyses of the six tests with total variance of 6, of which the greater part is in the six specifics. The sums of the communalities[5] in the two respective solutions were 3.5400 and 3.3230.

The solutions I_0, II_0, III_0 and I_0', II_0' and III_0' being sets of conjugate radii in two ellipsoids may be rotated into the axes of their respective ellipsoids.[6] The results are

SOLUTION	f_1	f_2	f_3	f_4	f_5	f_6	VARIANCE
First	0.6665	0.6434	0.0416	0.1559	0.0282	0.2662	0.9558
First	−0.2837	0.3956	−0.0728	−0.1454	0.4345	−0.1954	0.4904
First	0.3395	−0.2157	−0.0668	−0.2008	0.2381	−0.2260	0.3144
Second	0.6408	0.6097	0.0476	0.1681	−0.0106	0.4302	0.9980
Second	0.0069	0.4705	−0.0704	−0.1898	0.2114	−0.5900	0.6551
Second	0.1221	−0.0466	0.0907	0.1347	−0.2650	−0.1849	0.1478

It thus appears that in the first set a general may be found which has a total known variance of 0.9558 whereas there is a general with a known variance of as little as 0.3144; and in the second set a general may be found with a known variance as high as 0.9980 whereas one[7] may also be found with a variance as little known as 0.1478.

It is clear in the above solutions that the first (largest) vectors of the two solutions are more or less parallel, though of different length, whereas the other pairs of v ctors seem far from parallel. One may ask the question: How near together in direction may two generals, one from the first solution and the other from the second, be—or, per contra, how far divergent may they be? We cannot answer this for the generals themselves, which are unknown, but only for the team tests. The answer after a considerable amount of straightforward arithmetic is that the two generals whose team tests are nearest collinear[8] are separated by an angle of only $0^0.8$, whereas those whose team tests are most divergent include an angle of $80^0.5$, being thus nearly perpendicular. The two generals of that pair which is most nearly collinear are, however, not equally well known, the known variance of that which belongs to the first set of communalities is 0.9200 and of that which belongs to the second set of communalities is 0.8917.

Psychologists appear to determine the meaning of their generals, and thus to assign names to them by studying, the loading coefficients of the respective tests for the different generals, i.e., the projections of the tests upon the generals, and by comparing these with the psychological content of the tests.[9] They do not appear ordinarily to go on to the point of finding how well determined the factors are as judged by their known variances. Spearman did, to be sure, lay considerable stress on how well g was known and presumably was interested in enlarging his hierarchical system chiefly to get better determined estimates of g. It is easy to calculate from Thurstone's tables 7 and 3 (loc. cit.,[9] pp. 98 and 96, resp.) the variances of the known parts of his nine primary abilities,[10] viz.,

S(spatial), 0.61;	P(perceptual) 0.62;	N(number), 0.82;
V(verbal), 0.66;	M(memory), 0.43;	W(fluency), 0.44;
I(induction), 0.40;	R(reasoning), 0.60;	D(deduction), 0.52.

It is clear that considerable progress has still to be made before ratings on these primary abilities can be assigned to the individuals tested with a high degree of assurance.

[1] Wilson, E. B., and Worcester, J., these PROCEEDINGS, **25**, 73–77 (1939). If one may "count constants," there is a certain amount of generalization of the result which is indicated. Let M be the minimum number of communalities which may occur in a determinant of order $k + 1$. Then for n tests and k factors there is the important relation $M = 2k + 2 - n$, with the understanding that $M = 0$ means that some determinant of order $k + 1$ does not contain any communality (gives a relation between the correlation coefficients which must hold if the resolution is to be possible) and that there are determinants which contain only one communality (and can be used for its determination). For $M \gtrless 0$ we must have $n \lesseqgtr 2k + 2$. The case $n = 2k + 1$ gives $M = 1$ and will lead to a single analytical solution, although this will have no psychological significance unless all the communalities are positive proper fractions. We note that $T = {}^1/_2n(n - 1) - nk + {}^1/_2k(k - 1)$, which gives the number of independent conditions in the correlation coefficients for the resolution of n tests into k generals, has the value ${}^1/_2k(k - 1)$ and indicates relations between the coefficients in all cases except $k = 1$ (three tests and one general). For $M = 2$ we have $n = 2k$ and $T = {}^1/_2k(k - 3)$ and this indicates conditions on the correlation coefficients except when $k = 3$ (which appears to be the minimum value of k which has any interest and is indeed that of $n = 6$ now under discussion). However, there are indicated a whole series of cases $k = 4$, $n = 8$, ... for which there would be two quadratic equations in a pair of communalities with two analytic solutions and which would therefore probably lead to conclusions similar to that for $k = 3$, $n = 6$, namely, a pair of analytically distinct solutions, and cases of not too dissimilar matrices of correlation coefficients for which there would be two, one or no solutions with all communalities positive proper fractions.

So, too, with $M = 3$ we should have $n = 2k - 1$, $T = {}^1/_2(k^2 - 5k + 2)$. If $k = 4$, $n = 7$ and $T = -1$ the solution involves an arbitrary parameter as well as being analytically double. The first interesting case would seem to be $k = 5$, $n = 9$, $T = 1$ which involves but a single relation between the correlation coefficients. To determine the communalities we have then $M = 3$ simultaneous cubic equations in three of the communalities. There is, however, only a single cubic term and that is common to the three equations, so that by subtraction the system of three equations in three unknowns becomes one cubic and two quadratics which should have 12 solutions; but the type of the equations is such that 6 of these solutions are "at infinity." To illustrate we may consider the matrix

h_1^2	0.3750	0.1875	0.3750	0.3750	0.3750	0.1875	0.1875	0.0000
0.3750	h_2^2	−0.2500	−0.1250	0.0625	−0.1250	0.1250	−0.0625	−0.5625
0.1875	−0.2500	h_3^2	0.1875	0.0625	0.7500	−0.5000	0.1875	0.1875
0.3750	−0.1250	0.1875	h_4^2	0.3125	0.2500	0.1875	0.5000	0.1875
0.3750	0.0625	0.0625	0.3125	h_5^2	0.1875	0.1250	−0.3125	0.2500
0.3750	−0.1250	0.7500	0.2500	0.1875	h_6^2	−0.0625	0.3125	0.4375
0.1875	0.1250	−0.5000	0.1875	0.1250	−0.0625	h_7^2	0.3125	0.4375
0.1875	−0.0625	0.1875	0.5000	−0.3125	0.3125	0.3125	h_8^2	0.1250
0.0000	−0.5625	0.1875	0.1875	0.2500	0.4375	0.4375	0.1250	h_9^2

This matrix is resoluble into 5 generals and 9 specifics. For the three communalities h_4^2, h_5^2, h_6^2 one finds the six sets of values

$$h_4^2 = 0.8125,\ 0.3799,\ 0.5300,\quad 1.9681,\quad 0.0092 \pm 0.0489\sqrt{-1},$$
$$h_5^2 = 0.6875,\ 0.9945,\ 0.3777,\quad -2.6503,\quad -0.0371 \pm 0.1143\sqrt{-1},$$
$$h_6^2 = 0.8750,\ 0.2358,\ 0.9109,\quad 1.3895,\quad 0.4928 \pm 0.0845\sqrt{-1}.$$

Of these the last two, being complex, are useless and the fourth, lying outside the range of positive proper fractions, is equally useless; the first is in fact usable because all the other communalities turn out to be possible values; the other two solutions have not been examined to ascertain whether the remaining communalities are or are not possible

values. The principle is what we desire to illustrate, namely, that there are six analytical solutions. It is not impossible therefore that a 9×9 matrix could be found which was analyzable in any number of ways from 0 to 6 inclusive into 5 generals plus specifics, and, further, that it might be possible to find 7 matrices which did not markedly differ and which were respectively so analyzable in 0, 1, ..., 5, 6 distinct ways; and finally such possibilities would appear to be involved in any complete discussion of the resolution of $2k - 1$ tests into k factors.

Kelley, T. L., *Crossroads in the Mind of Man*, p. 104, analyzes $n = 9$ tests into $k = 6$ factors. Here the value of T is -3 so that the resolution involves 3 parameters, and $M = 5$ so that the problem of determining the communalities would involve simultaneous quintic equations in five unknowns and while it might lead to only a single 3-parametered solution, might apparently lead to a considerable number of such solutions—or to none because none would come out real (as Kelley's fails to do). Again, on p. 122, Kelley has the problem of the resolution of $n = 13$ tests into $k = 7$ factors. Here $T = 8$ and for a strict mathematical solution 8 independent conditions must be satisfied between the correlation coefficients. Assuming these satisfied, $M = 3$ and there is the possibility of any number of distinct solutions from 0 to 6 inclusive. (We are aware of the fact that Kelley has abandoned this type of multiple factor analysis and adopted the Hotelling type—see his *Essential Traits of Mental Life.*) Thurstone seems to deal only with cases in which n is so much larger than k (to the degree of approximation to which he pushes his analysis) that M is negative and the question of multiple solutions does not arise. This is as though in the analysis of 6 tests we had been content with getting out two factors in which case the whole work would have been approximate, but to what degree one could hardly tell *a priori*.

[2] The authors are indebted to the Carnegie Corporation for a grant to cover the cost of the calculations involved.

[3] Wilson, E. B., these PROCEEDINGS, **20**, 193–196 (1934).

[4] The calculations had to be carried to a good many places in order to keep the arithmetical work checking fairly satisfactorily but in giving the results the numbers are cut down in order to save space. We are keeping to the notation of the previous paper.[1] From some points of view it might have been better to determine the set of orthogonal unit vectors that would constitute a Hotelling analysis of the six tests, but the method used seemed in other ways simpler.

[5] Thomson, G. H., *Brit. J. Educ. Psychol.*, **8**, 255–264 (1938), and *The Factorial Analysis of Human Activity*, Chap. viii (1939), emphasizes the maximization of the specifics by Thurstone's analysis. In view of our findings here we feel somewhat doubtful as to the precise meaning to be attributed to this statement of Thomson's.

[6] The coefficients are orthogonal, it is really the vectors I, II, III which are rotated.

[7] Note that the three axes of the ellipsoids correspond to a possible set of three generals, i.e., there are three generals which remain perpendicular in their projections. If we adopt Thurstone's point of view that none of the generals which may be found means anything psychologically except as it is proved to mean something, and the further implied assumption that some set of generals does mean something psychologically, we may not assume that this particular set of generals whose team tests are uncorrelated does mean anything; to have meaningful generals it may be necessary that the team tests be correlated. Thurstone's assumption deserves a comment. When in a mathematical system we find parameters available (as in the rotation of a set of orthogonal generals) we may proceed, as he does, by *specification* to state that there is meaning only for certain values of the parameters, or we may say that there is meaning for any set of the parameters (but with different meanings for different sets), or finally we may proceed by *abstraction* to say that there is meaning only in the total group. In the

first sense only some particular set of three generals, in so far as defined by three conjugate radii of an ellipsoid in the 6-space of the tests, has meaning; in the second any such set has meaning; in the third it is only the unit sphere in the (unknown) space of the generals, or in so far as can be known from the given tests, the ellipsoid defined by the projection of this sphere which has meaning—i.e., there are no meaningful factors but only a meaningful factor complex.

[8] The team tests are respectively

$$t = 0.6808f_1 + 0.5929f_2 + 0.0686f_3 + 0.2208f_4 - 0.1059f_5 + 0.3469f_6,$$
$$t' = 0.6805f_1 + 0.5935f_2 + 0.0800f_3 + 0.2141f_4 - 0.1045f_5 + 0.3480f_6.$$

[9] See, e.g., Thurstone, L. L., *Primary Mental Abilities*, Chap. V.

[10] We obtained the table of values from tables 7 and 3 as stated; whether the result is a fair estimate of the determination of the abilities we cannot state with certainty. The path pursued by Thurstone was devious. He started with 57 tests and performed a factor analysis (of his approximate type) upon them, proceeding to the extent of extracting 12 factors; the total space in which one is working theoretically is of 217 (?) dimensions (there being 218 (?) subjects or somewhere between 218 and 240). In this space lie the 57 unit test vectors which are ideally to be resolved into a certain number of generals and 57 specifics. Unless there are relations between the correlation coefficients the number of generals would be in the neighborhood of 47 (for which $T = -2$ and denotes 2 parametric degrees of freedom) so that there would be involved for the 57 specifics and the 47 generals a space of 104 dimensions included in that of 217. The 12 factors isolated taken with the 57 specifics constitute a space of 69 dimensions which is not a subspace of that 104 just mentioned but is (we take it) supposed to be nearly a subspace thereof. All that can be known of the 12 generals is their projections on the 57-dimensional space of the tests, and these projections are supposed (apparently) to be nearly congruent with those of the true 12 factors of highest (or nearly highest) variance that would be extracted from the 57 tests by a complete and accurate analysis. However, these 12 factors are meaningless psychologically until they have been rotated (in their own space of 12 dimensions) and Thurstone thus rotates them (using his principle of simple structure as a guide). It may be noted at this point that had the analysis been pushed to 15 or 20 factors, these 15 or 20 would have been rotated in *their* space, the rotation would not have been in the subspace of 12 dimensions defined by the original 12 factors and it appears not to have been proved that the results would have been essentially the same for any 12 factors (though with the vast experience Thurstone has had with his analysis he may have the feeling and may be right in having the feeling that such would be the case). Returning to the 12 factors isolated, Thurstone picks out certain combinations of tests, using in all only 18 of the original 57, which are to define anew at least approximately his 9 primary abilities directly and without any new factorial analysis or rotations and he computes his table 3 (p. 96) of the intercorrelations of these not from their definitions in terms of the new test-combinations but from the resolutions of these tests along the 12 afore-determined factors. (These intercorrelations appear to be much the same as those that would have been obtained directly—the most notable exception being the value 0.20 obtained for the correlation of C_S with C_D which computed directly would seem to be 0.29.) If the whole complicated or, as we have said, devious procedure is to be regarded merely as a heuristic aid in arriving at the group of 9 test-combinations and if the justification for the existence of the 9 primary abilities is to inhere in the *a posteriori* discussion and examination of them, as thus finally found, in relation to individual characteristics of the persons tested and in relation to the importance of these characteristics in the mental constitution of the individuals; if in short the psychological work has but begun when the 9 test-combinations have been found, then there can be no exception taken to the procedure, however

complicated or devious it may appear—no more than one could take exception to the apparently more direct procedure of Thorndike in defininig intelligence by his CAVD. What seems, however, to be implied by the emphasis upon the complicated procedure as a basis for determining primary abilities is that there is something inherently sound in it which by that soundness leads to a definition of the abilities. In view of the approximative nature of the whole process we should apparently need from a logical point of view some mathematical theorems in approximations which have not yet been demonstrated to prove that the results obtained do not differ too greatly from those that would be obtained by other similar procedures applied to other groups by other persons looking toward the same general psychological ends. Concerning this matter we have no judgment; what we have tried to do in this and previous papers is to throw some light on the mathematical implications of the conceptual set-up of factor analysis itself as a methodology.

INDEPENDENT GENERATORS OF THE SUBGROUPS OF AN ABELIAN GROUP

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The subgroups of an abelian group are known to be the direct products of their Sylow subgroups whenever their orders are not powers of a prime number. Hence we shall confine our attention in the present article to abelian groups whose orders are of the form p^m, p being a prime number. The orders of the subgroups under consideration are therefore also of this form. The simplest case is the one in which all the invariants of such a group, G, are equal to each other. Let p^{m_1} represent such an invariant and hence m/m_1 is an integer which will be represented by k. Since all the operators of highest order in an abelian group are conjugate under its group of isomorphisms we can select any operator of highest order contained in G as an operator of one of its sets of independent generators. The number of ways of choosing the first operator of such a set is therefore $p^{km_1} - p^{k(m_1-1)}$.

After the first operator of this set has been chosen the second operator thereof can be selected arbitrarily from the operators of highest order in G which do not generate the same subgroup of order p as is generated by the selected independent generator. The number of the operators of order p^{m_1} in G whose p^{m_1-1} power is the same operator of order p is $p^{k(m_1-1)}$ since it is the product of one such operator into all the operators of G whose orders divide p^{m_1-1}. The number of ways of selecting a second operator of a set of independent generators of G is therefore

$$p^{km_1} - p^{k(m_1-1)} - (p-1)\,p^{k(m_1-1)} = p^{km_1} - p^{k(m_1-1)+1}.$$

When $k > 2$ we may choose for a third operator in the given set of independent generators of G any one of the operators of order p^{m_1} contained in G except those which generate one of the $p^2 - 1$ operators of order p in the group generated by the two independent generators of G which have already been selected. Such a third operator in a set of independent generators of G can therefore be chosen in $p^{km_1} - p^{k(m_1-1)+2}$ ways. As similar remarks apply to the selection of the other independent generators, the kth operator of the given set of independent generators of G can be chosen in $p^{km_1} - p^{k(m_1-1)+k-1}$ ways whenever $k \geqq k_1$. The number of ways of selecting all the operators of a set of independent generators of G is equal to the order of the group of isomorphisms of G.

If a subgroup of G has λ invariants all of which are equal to p^α but has no other invariants it appears in the subgroup of G which is composed of all the operators of G whose orders divide p^α. This subgroup has k invariants which are separately equal to p^α and it has no other invariants. A set of its independent generators can therefore be selected from the operators of G in $(p^{k\alpha} - p^{k(\alpha-1)})(p^{k\alpha} - p^{k(\alpha-1)+1}) \ldots (p^{k\alpha} - p^{k(\alpha-1)+\lambda-1})$ ways. The number of these subgroups contained in G is the quotient of the two products noted in this paragraph. By dividing each term of the former of these products by $p^{k(\alpha-1)}$ and each term of the latter by $p^{\lambda(\alpha-1)}$ there results the quotient

$$\frac{(p^k - 1)(p^k - p) \ldots (p^k - p^{\lambda-1})}{(p^\lambda - 1)(p^\lambda - p) \ldots (p^\lambda - p^{\lambda-1})}$$

which is known to represent the number of subgroups having λ invariants contained in the abelian group of type 1^k.

From the formulas developed in the preceding paragraph the following theorem can be deduced: *If an abelian group of order* p^{m}, p *being a prime number has all its invariants equal to each other and the number of these invariants is* k *then the number of its subgroups which also have all their invariants equal to each other and have* λ *such invariants which are equal to* p^α *is equal to the product of the number of the subgroups of type* 1^λ *in the group of type* 1^{k}, *and* $\mathrm{p}^{\lambda(\mathrm{k}-\lambda)(\alpha-1)}$. This theorem reduces the determination of the number of the subgroups which have equal invariants and appear in a group which has equal invariants to the determination of the number of the subgroups of the group of type 1^k which have a given number of invariants. As the latter problem has been completely solved the former is also solved.

If an abelian group of order p^m has different invariants it is the direct product of subgroups such that all the invariants of each of these subgroups are equal to each other. It should be emphasized that the given formula to determine the number of the subgroups which have all their invariants equal to each other and appear in a group which has a similar property was noted above on account of its simplicity. Theoretically the

problem of determining the number of the subgroups of a given type which appear in a general abelian group has been solved since it is only necessary to divide the number of ways in which a set of independent generators of such a subgroup can be selected from the operators of the group by the number of ways in which such a set can be selected from the operators of the given subgroup. The general formula for determining this quotient is, however, complex and hence its explit form may not be of much interest.

In special cases the corresponding formula becomes quite simple. For instance, to determine the number of the subgroups of an abelian group of order p^m which have for their invariants the orders of all the operators of largest order contained in a set of independent generators of the group it is only necessary to observe that each operator of a set of independent generators of such a subgroup can be selected from the operators of the group in a number of ways which is equal to the number of ways in which it can be selected from the operators of the subgroup multiplied by the product of the orders of all the operators of lower orders which appear in the given set of independent generators of the group. This may be expressed in the form of a theorem as follows: *The number of the subgroups of a group of order* p^m, p *being a prime number, which separately have for their invariants the orders of all the operators of highest order in a set of independent generators of the group is equal to the product of all the orders of the operators which are not of largest order in the given set of independent generators of the group raised to a power which is equal to the number of operators of largest order in the given set of independent generators of the group.*

This theorem can be generalized by noting that the invariants of the subgroups in question need not be confined to the orders of the largest operators in a set of independent generators of a group but may include the orders of all the operators of highest order, next to the highest order, etc. The number of such subgroups is then equal to the product of all the orders of the remaining operators in the given set of independent generators of the group raised to a power whose index is equal to the sum of the number of the operators of the largest order, of the next largest order, etc. For instance, if a given group has a set of independent generators which involves five operators of highest order, three operators of next to the highest order, four operators of the next order, and if the product of the orders of the remaining operators in the given set of independent generators is 16, then the number of its subgroups which separately have five invariants which are equal to these operators of highest order, three invariants which are equal separately to the operators of next to the highest order in the given set of independent generators and four invariants which are separately equal to the next orders is 16^{12}.

Suppose that a subgroup of G has for its invariants the orders of all the operators of lowest order in a set of independent generators of G. The

number of such subgroups is clearly the same as the number of the subgroups having the same invariants in the subgroup of G composed of all of its operators whose orders do not exceed this invariant. Hence the number of these subgroups is the number of the subgroups having equal invariants contained in a group whose invariants are all equal to each other and results from the first italicized theorem of the present article. To determine the number of the subgroups of G, when G has unequal invariants, which have for their invariants the orders of the operators of the two lowest orders in a set of independent generators of G it may be noted that the operators of the larger order in a set of independent generators of such a subgroup may first be selected from the operator of the group and then from the operators of the subgroup, as if the operators of the smaller order did not appear, since they do not affect the quotient which determines the number of these subgroups.

After the independent generators of the larger order have been chosen, those of the smaller order can again be selected as in the case when all the invariants of a group are equal to each other and all the invariants of the subgroups in question have the same property. Similar remarks apply to all cases in which the subgroups in question have for their invariants all the orders of the operators in a set of independent generators of G which do not exceed a given number. Hence the determination of the number of such subgroups is also reduced to the first italicized theorem of the present article.

GROUPS HAVING A SMALL NUMBER OF SUBGROUPS

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The term subgroup is used in the present article in a restricted sense, excluding both the identity and the group itself. Such a subgroup has sometimes been called a proper subgroup and this term will be used here occasionally for the sake of emphasis. It is well known that the group of order p, where p is any prime number, is the only group which does not contain a proper subgroup and that every cyclic group of order p^m has exactly $m - 1$ subgroups for every value of the prime number p, whenever $m > 1$. Hence there is always an infinite number of distinct groups which separately have the property that each of them contains an arbitrarily given number of proper subgroups if two groups of different numerical orders are regarded as necessarily distinct.

When a group has one and only one proper subgroup it must belong to this infinite system of prime power cyclic groups. On the other hand, if a group has two and only two proper subgroups it is either the cyclic group of order p^3 or the cyclic group whose order is the product of two distinct prime numbers. Hence it belongs to either of two infinite systems of well-known cyclic groups and it is an arbitrary one from either of these two systems. If a group has three and only three proper subgroups it may be the well-known four group and hence it is not necessarily cyclic. It is, however, still necessarily abelian since a non-abelian group in which every subgroup is invariant is known to be Hamiltonian and hence it is the direct product of the quaternion group and an abelian group. It therefore involves at least four proper subgroups. If a group contains a non-invariant subgroup it will be seen that it contains at least four proper subgroups.

It should be noted that *if a group* G *has exactly* n *proper subgroups these cannot be transformed under* G *according to a substitution group which involves a substitution of degree* n. In particular, they cannot be transformed transitively under G. If the non-abelian group G had exactly three proper subgroups two of them would be conjugate according to this theorem and they would be transformed according to the group of order 2 and of degree 2. They would necessarily correspond to the identity of this group and they would be of prime order. Their product would be of the same order since they are commutative and of the same prime order. As this is impossible it results that *a non-abelian group contains at least four proper subgroups and if an abelian group contains exactly three proper subgroups it is either the four group or the cyclic group of order* p^4. The latter part of this theorem results directly from the fact that such an abelian group could not involve more than one Sylow subgroup.

The dihedral group of order $2p$ contains exactly $p + 1$ proper subgroups. Hence there are at least two non-abelian groups which separately involve exactly four proper subgroups, viz., the quaternion group and the symmetric group of degree 3. It is easy to prove that these are the only non-abelian groups which have this common property. For if a Hamiltonian group is not the quaternion group it contains at least ten proper subgroups and if G is non-abelian and contains exactly four proper subgroups these subgroups cannot be transformed under G according to a group of degree 4 since each of these groups involves a substitution of degree 4. Hence at least one of these four subgroups is invariant under G. If three are conjugate, G is the symmetric group of order 6 since it cannot be the tetrahedral group. If two of the four subgroups would be invariant all would be invariant and G would be the quaternion group because the product of two would be invariant.

If G is abelian and contains exactly four proper subgroups its order is

not divisible by more than two prime numbers and if its order is divisible by two such numbers it is of the form $p_1p_2^2$, p_1 and p_2 being prime numbers and the Sylow subgroup of order p_2^2 is cyclic; if its order is the power of a prime number it is cyclic and of order p^5 or the noncyclic group of order 9. This completes a proof of the following theorem: *There are two and only two non-abelian groups which separately contain as few as four proper subgroups. One of these is the quaternion group and the other is the symmetric group of order* 6. *If an abelian group contains four proper subgroups it is the non-cyclic group of order* 9 *or a cyclic group whose order is either* p^5 *or* $p_1p_2^2$, p_1 *and* p_2 *being prime numbers.*

If an abelian group has exactly five proper subgroups its order cannot be divisible by as many as three distinct prime numbers since the number of these prime numbers increased by the number of their pairs is at least 6. It cannot be divisible by two distinct primes since the Sylow subgroups would be cyclic. Hence it results that the only abelian group which has exactly five proper subgroups is of order p^6 and belongs to the system noted in the first paragraph of this article. In fact, this is the only group which has exactly five proper subgroups since it will be proved in what follows that no non-abelian group whatever has this property.

Suppose that the non-abelian group G contains five subgroups. If these were transformed according to a group of degree 5 it would be intransitive and of order 6. As this case is impossible G would involve at least one invariant subgroup. The other four subgroups could not be conjugate or appear in two sets of two conjugates and hence this case is also impossible. Since G could not contain as many as two invariant subgroups it results that *no non-cyclic group can contain exactly five proper subgroups and if a cyclic group contains exactly five proper subgroups it is of order* p^6, p *being a prime number.*

An abelian group which contains exactly six proper subgroups and has an order which is divisible by three distinct prime numbers is cyclic and its order is not divisible by the square of a prime number. If its order is divisible by two distinct prime numbers it must be the form $p_1p_2^3$, p_1 and p_2 being prime numbers, and the group is cyclic. Finally, when it is a prime power group it is one of the following three groups: The group of order 8 and of type 2, 1, the non-cyclic group of order 25, the cyclic group of order p^7. Hence there are five abelian groups which separately involve six proper subgroups. Three of these consist of infinite systems and are cyclic, while the other two are individual groups. We proceed to determine the non-abelian groups which separately involve exactly six proper subgroups.

It was noted above that the dihedral group of order $2p$, p being a prime number, contains $p + 1$ proper subgroups and hence it contains six such subgroups when $p = 5$, and it is obvious that when G contains six proper

subgroups such that as many as five of these are conjugate under G then it must be this dihedral group. When G contains exactly six proper subgroups they cannot appear in two sets of conjugates involving four and two subgroups, respectively, since the corresponding intransitive group of degree 6 would involve a substitution of degree 6. For a similar reason G could not have two sets of conjugates each being composed of three subgroups. If G contained three sets of conjugates each set being composed of two subgroups it would transform these subgroups according to the intransitive group of order 4 and of degree 6 which is composed of positive substitutions and hence it would involve an invariant subgroup corresponding to the identity of this intransitive group and involving at most two proper subgroups. As such a group would involve at least one invariant subgroup, this is impossible.

From the preceding paragraph it results that if G involves exactly six proper subgroups at least one of them is invariant, and if only one of them is invariant the rest cannot be transformed transitively unless G is the dihedral group of order 10. If the rest were transformed according to an intransitive group whose transitive constituents are of degree 3 and 2, respectively, this intransitive group could not be of order 12 since G involves only 6 subgroups. Hence it would be of order 6. It could not be the cyclic group of this order since the subgroup of G corresponding to the identity of this cyclic group would involve at most two proper subgroups. This is impossible because these subgroups would be invariant. For similar reason the corresponding intransitive subgroup could not be non-cyclic. If three of the subgroups of G are invariant while the other three are conjugate then G is the dicyclic group of order 12 since the three conjugate subgroups could not be transformed according to the group of order 3.

If G would involve two sets of two conjugate subgroups they could not be transformed according to the intransitive group either of order 4 or of order 2. Similarly it may be seen that G could not involve four invariant subgroups and one set of two conjugate subgroups. Hence there results the following theorem: *If a non-abelian group contains exactly six proper subgroups it is either the dihedral group of order* 10 *or the dicyclic group of order* 12. *If an abelian group contains exactly six proper subgroups it is one of the following five groups: The group of order* 8 *and of type* 2.1, *the non-cyclic group of order* 25, *the cyclic group whose order is divisible by three distinct prime numbers but not by the square of a prime number, the cyclic group of order* $p_1p_2^3$, p_1 *and* p_2 *being distinct prime numbers, the cyclic group of order* p^7.

If an abelian group contains exactly seven subgroups its order cannot be divisible by three distinct prime numbers and if it is divisible by two prime numbers it is the cyclic group of order $p_1^2p_2^2$, p_1 and p_2 being distinct

prime numbers. If its order is a power of a single prime it is the cyclic group of order p^8. Hence it results that if an abelian group involves exactly seven proper subgroups it is an arbitrary one of two infinite systems of such groups. To prove that a non-cyclic group does not contain exactly seven subgroups it may first be noted that it could not transform these subgroups according to a group of degree 7 because it would involve at least one invariant subgroup. It could also not transform them according to a group of degree 6 since it could not involve just one invariant subgroup. As it could not transform its non-invariant subgroups according to a group of degree 5 it would have to involve at least three invariant subgroups.

Suppose that G contains exactly three invariant subgroups. The four non-invariant subgroups would then be transformed according to a group of degree 4. This could not be the four group since G is supposed to contain only three invariant subgroups. It could not be the cyclic group of order 4 since G could not be of order 36. It could not be of order 2 since G could not involve a subgroup of index 2 which would be either abelian or Hamiltonian. If G contained three non-invariant subgroups they would be transformed transitively under G. As this is impossible and as G could not contain exactly two non-invariant subgroups it results that *there is no non-cyclic group which has exactly 7 proper subgroups, and the cyclic groups of order* p^8 *and of order* $p_1^2p_2^2$, p_1 *and* p_2 *being prime numbers, are the only cyclic groups which have this property.*

ON CHAIN-MAPPINGS CARRIED BY CELL-MAPPINGS

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An abstract complex,[1] X, is characterized by an additive set-function, C, called *closure* and a linear chain-function, F, called *boundary*. C assigns to each cell x of X a set of cells Cx of X such that $x \in Cx$ and $CCx = Cx$. F assigns to each (oriented) cell x of X a chain Fx of dimension $p - 1$ ($p = \dim x$) such that Fx lies on Cx and $FFx = 0$. For simplicity we shall use chains with integral coefficients and assume that our complexes are finite. By a *cell-mapping* S of X in a complex X' we mean an additive set-function which assigns to each cell x of X a set Sx of cells of X' such that

$$CSx = CSCx. \tag{1}$$

By a *chain-mapping* T *carried by* S we mean a linear chain-function which assigns to each cell x of X a p-chain Tx of CSx such that

$$FTx = TFx. \tag{2}$$

Let S carry a second chain-mapping T_1. Then we say that T_1 is *equivalent to* T ($T_1 \sim T$) *over* S if there is a linear chain-function D which assigns to each cell x of X a $p + 1$-chain Dx of CSx such that

$$FDx = Tx - T_1x - DFx. \tag{3}$$

The point of this equivalence is that under (3) T and T_1 map any cycle c of X into cycles which are homologous on $CS|c|$, where $|c|$ is the set of cells carrying c. Of course if Sx does not raise dimensions, i.e., if no Sx contains cells of dimensions greater than $p = \dim x$, then the equivalence $T_1 \sim T$ over S implies $T_1 = T$. Let Y be a closed subcomplex of X. Then replacing "to each cell x of X" by "to each cell x of Y" in the two chain-mapping definitions given above, we have the slightly more general notions of (*a*) T carried by S *defined on* Y and (*b*) $T_1 \sim T$ over S *relative to* Y.

We notice that (2) lends itself to an inductive definition of T as $\dim x = p$ ascends. Assuming TFx known and $FT(Fx) = TF(Fx)$, Tx can be defined as a chain bounded by TFx. Due to (1) TFx must lie on CSx. So it is sufficient for a step of the induction to know that each p-1-cycle on CSx bounds on CSx. In the same way (3) lends itself to an inductive definition of D. Assuming DFx known and $FD(Fx) = T(Fx) - T_1(Fx) - DF(Fx)$, Dx can be defined as a chain bounded by $Tx - T_1x - DFx$. Due to (1) the latter must lie on CSx. So it is sufficient for a step in the induction to know that each p-cycle (if any) on CSx bounds on CSx. To start either induction we must assume knowledge of T or D (and (2) or (3) of course) on some closed subcomplex Y. We accordingly have the following two theroems.

THEOREM 1. *If* S *is such that for* x ϵ X-Y *each* p-1-*cycle of* CSx *bounds on* CSx, *and if* S *carries* T *defined on* Y, *then the definition of* T *can be extended to all of* X *with* S *as carrier.*

THEOREM 2. *If* S *is such that for* x ϵ X-Y *each* p-*cycle of* CSx *bounds on* CSx *and if* S *carries* T *and* T_1 *so that* $T \sim T_1$ *over* S *relative to* Y, *then* $T \sim T_1$ *over* S *for all of* X.

The extension of T established in Theorem 1 is not necessarily unique since TFx may bound more than one chain of CSx, but if Theorem 2 also holds the extension is unique within homology. Hence the following theorem.

THEOREM 3. *If* S *is such that for* x ϵ X-Y *each* p-1 *and* p-*cycle of* CSx *bound on* CSx, *and if* S *carries* T *defined on* Y, *then* T *can be extended to all of* X *with* S *as carrier, and this extension is unique within homology.* (*If* S *does not raise dimensions, the extension is unique.*)

Let X' contain X and let $x \in CSx$ for each x of X. Then S carries the identical chain-mapping $Ix = x$. So, applying Theorem 2, we have:

THEOREM 4. *If* S *is such that* x ϵ CSx *and such that for* x ϵ X-Y *each* p*-cycle of* CSx *bounds on* CSx, *and if* S *carries* T *such that* T $\sim$ I *over* S *relative to* Y, *then* T $\sim$ I *over* S *for all of* X.

Let S be a cell-mapping of X in X' and let S' be a cell-mapping of X' in X''. Then $S'S$ is a cell-mapping of X in X'' since $CS'Sx = CS'CSx = CS'CSCx = CS'SCx$. Let S carry T and let S' carry T'. Then $S'S$ carries $T'T$ since $FT'Tx = T'FTx = T'TFx$ and $T'Tx$ is a chain of $CS'CSx = CS'Sx$. If X'' contains X and $S'S$ and $T'T$ satisfy the requirements of Theorem 4 we have that $T'T \sim I$. The point of the latter is that it shows that the homology groups of X are (simply) isomorphic to subgroups of the corresponding homology groups of X'.

The inverse S^{-1} of a cell-mapping S of X in X' does not necessarily satisfy the requirements for a cell-mapping of X' in X. However, when S is one-many and maps X *on* X' (i.e., the mapping covers all of X'), then S^{-1} is a cell-mapping defined on X' if and only if $CSx = SCx$. This leads to a definition[2] of subdivision for general abstract complexes and to the theorem which follows.

DEFINITION. We say that a mapping S *subdivides* X into X' if S is a one-many mapping of X on X' such that $CSx = SCx$, and if S and S^{-1} carry chain-mappings T and T' such that $T'T = I$ and $TT' \sim I$ over SS^{-1}.

THEOREM 5. *If* S *is a one-many mapping of* X *on* X′ *such that* CSx = SCx *and such that for* x ϵ X-Y *each cycle of* Cx *and* CSx *bounds on* Cx *and* CSx, *and if* S *subdivides* Y *into* Y′ = SY, *then* S *subdivides* X *into* X′.

If the mapping S which subdivides X into X' is one-to-one then $TT' = I$ and so S^{-1} subdivides X' into X. In this case we can regard X' as a copy of X with cells reoriented by T.

APPLICATIONS.—A simple application of the preceding theory occurs when the closed subcomplexes Y and CSY are single cells. Then, subject to the requisite conditions regarding cycles of CSx bounding on CSx, a chain-mapping T carried by S is determined within homology by its definition on the single cell Y. If $x \in CSx$ then any chain-mapping T carried by S is equivalent to I over S provided $T = I$ for the single cell Y. And so on.

Let K be a simplicial complex, or a complex composed of convex cells, which is "closed" in the sense that if any cell belongs to K so do all its faces. (Or, more generally, K may be any abstract complex possessing "closure uniformity."[1]) Then it is possible to adjoin to K an ideal cell i of dimension -1 so that $X = K + i$ is a complex in which $Fx = i$ for each 0-cell x of K. If x is a cell ($\neq i$) of X then any cycle of Cx bounds on Cx. This provides an excellent opportunity to apply our theorems using $X = K + i$ and $Y = i$. We notice in particular that any T carried by a single-valued cell-mapping S of $X = K + i$ in $X' = I' + i'$ is fully determined by its

definition on the single cell i. Simplicial mappings illustrate this property.

Let K have added to it an "open fringe"[1] J such that $K + J$ is a complex in which any cycle of the closure in $K + J$ of a cell of J bounds on that closure. Now we cannot augment $K + J$ by i so as to have a complex. But by first taking $X = K + i$ and $Y = i$, and then in a second step taking $X = K + J$ and $Y = K$, it is possible under suitable conditions to characterize a chain-mapping T by means of a cell-mapping S which carries it.

Graphs and Products.—Let X^* denote the dual complex[1] of an abstract complex X, and let $X^* \times X'$ denote the product complex of X^* and X'. Then a cell-mapping of X in X' has a *graph* G in $X^* \times X'$ consisting of the set of all product-cells $x^* \times x'$ such that $x' \in CSx$. G is a closed subcomplex of $X^* \times X'$ if and only if S satisfies (1).

A chain-mapping T carried by S is represented on G by a chain which is a cycle[1] if and only if T satisfies (2). If T_1 is a second chain-mapping carried by S, then $T \sim T_1$ over S if and only if T and T_1 are represented by cycles homologous on G.

The dual graph G^* of S in the product $X \times X'^*$ is an open subcomplex of $X \times X'^*$. The mapping S_1 of X on G^* which assigns to each x of X the set $x \times (CSx)^*$ of cells of G^* is one-many and satisfies the condition $CS_1x = S_1Cx$. Hence under suitable conditions S_1 may subdivide X into G^* In particular, let $X = K$ and $X' = K'$ (where K and K' are as mentioned above) and let S be a single-valued mapping of X in X'. Then S_1 subdivides X into G^*. Hence there is a chain-mapping T' carried by S_1^{-1} which is unique within homology. The definition of T' can be formally extended throughout $X \times X'^*$ by the convention that $T = 0$ on the closed complement of G^*. Then $T'(x \times x'^*)$ is a chain of X in which the coefficient of any cell y is necessarily zero unless $x \in Cy$ and $x' \in CSy$. Hence T' yields a product theory depending only on S. For the special case $X' = X$ and $S = I$ this product reduces to the "cap product" of Whitney.[3]

The considerations of the last paragraph extend with suitable modifications to complexes $K + J$ as mentioned above.

[1] Cf. Tucker, *Ann. Math.*, **34**, 191–243 (1933).

[2] Tucker, *Rec. math. Moscou*, **1**, 773–774 (1936).

[3] Whitney, *Ann. Math.*, **39**, 397–432 (1938).

THE ANALYTIC PROLONGATION OF A MINIMAL SURFACE ACROSS A STRAIGHT LINE

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1. In a recent paper[1] (referred to hereafter as (I)) we proved a result which may be stated in the following form.

THEOREM. *Let* M *denote a minimal surface contiguous*[2] *to a straight line* l. *If* M *be rotated about* l *through* 180°, *thus giving a minimal surface* M′, *then* $\mathfrak{M} = \mathrm{M} + \mathrm{M}'$ *forms a single analytic minimal surface, regular where it traverses the line* l.

The purpose of this note is to present a shorter and more perspicuous proof of this theorem. Also, our assumption as to the relation of M to l is somewhat less restrictive than in (I).[3]

As stated in (I), the point of our theorem is that l is not assumed to be interior to M, but only to form part of its boundary. If l is interior to M, the theorem becomes a well-known one of Schwarz which states the symmetrical nature of a minimal surface with respect to any straight line which it may contain.

2. We take the line l in the position of the axis of z, which occasions no loss of generality since all our conditions are invariant under rigid motion.

The minimal surface M may be represented analytically in the form

$$x = \Re f_1(w), \qquad y = \Re f_2(w), \qquad z = \Re f_3(w), \tag{1}$$

$$f_1'^2(w) + f_2'^2(w) + f_3'^2(w) = 0, \tag{2}$$

where $w = u + iv$ ranges over the upper half-plane $v > 0$. The relation of M to l is expressed by the following condition: as $w \to u$, any fixed point of a certain interval $a < u < b$ of the u-axis,

$$x = \Re f_1(w) \to 0, \qquad y = \Re f_2(w) \to 0. \tag{3}$$

This means that as $w \to u$, the distance from l of the corresponding point $P(w)$ of M tends to zero, which does not of itself imply that $P(w)$ tends to any particular point of l. This behavior, not postulated for $P(w)$, will result, however, from our proof, since the functions (1) defined in $(v > 0)$ will be proved to be part of the functions (13) defined and regular in a domain S that includes ab. To this slight extent the result of this note is more general than that of (I), where the functions (1) were postulated to remain one-valued and continuous on ab.

Let S denote the slit-plane[4] $(v > 0) + ab + (v < 0)$. Our problem is to prove the existence of functions $\varphi_1(w)$, $\varphi_2(w)$, $\varphi_3(w)$ defined and analytic

throughout S and coinciding in $(v > 0)$ with $f_1(w), f_2(w), f_3(w)$, respectively. The relation

$$\varphi_1'^2(w) + \varphi_2'^2(w) + \varphi_3'^2(w) = 0 \tag{4}$$

throughout S will then be an immediate consequence of the fact that (2) holds in $(v > 0)$.

3. From (3) it follows by the Schwarz symmetry principle that the functions $f_1(w), f_2(w)$ have analytic prolongations $\varphi_1(w), \varphi_2(w)$ defined in S, which obey the relations

$$\varphi_1(\bar{w}) = -\overline{\varphi_1(w)}, \qquad \varphi_2(\bar{w}) = -\overline{\varphi_2(w)}, \tag{5}$$

where the bar denotes the conjugate complex quantity.

If $w = u(a < u < b)$, it results from (5) that $\varphi_1(u), \varphi_2(u)$ are pure imaginary. Hence $\varphi_1'(u), \varphi_2'(u)$ are pure imaginary, as we see by performing the differentiation in the direction of the real axis. As $w \to u$ from $(v > 0)$, we have then by (2)

$$f_3'^2(w) \to -\varphi_1'^2(u) - \varphi_2'^2(u) = p, \tag{6}$$

where p is a non-negative real number.

Keeping in mind that $+\sqrt{p}, -\sqrt{p}$ are real, we observe the inequalities

$$|\Im f_3'(w)| = |\Im\{f_3'(w) + \sqrt{p}\}| \leqq |f_3'(w) + \sqrt{p}|,$$
$$|\Im f_3'(w)| = |\Im\{f_3'(w) - \sqrt{p}\}| \leqq |f_3'(w) - \sqrt{p}|,$$

whence, by multiplication and the taking of the positive square root,

$$|\Im f_3'(w)| \leqq +\sqrt{|f_3'^2(w) - p|}. \tag{7}$$

It follows by (6) that

$$\Im f_3'(w) \to 0 \tag{8}$$

as $w \to u$ from $(v > 0)$.

Accordingly, by Schwarzian symmetry again, $f_3'(w)$ is part of a function $\varphi_3'(w)$ defined and analytic throughout S, which obeys the relation

$$\varphi_3'(w) = \overline{\varphi_3'(\bar{w})}. \tag{9}$$

We may now define throughout S

$$\varphi_3(w) = f_3(w_0) + \int_{w_0}^{w} \varphi_3'(w)dw, \tag{10}$$

w_0 denoting any fixed point in $(v > 0)$. Since $\varphi_3'(w) \equiv f_3'(w)$ for w in $(v > 0)$, we have $\varphi_3(w) \equiv f_3(w)$ in $(v > 0)$. In other words, $\varphi_3(w)$ is the analytic prolongation of $f_3(w)$ over the region S.

Writing

$$\psi(w) = \varphi_3(w) - \overline{\varphi_3(\bar{w})} \tag{11}$$

(an analytic function of w), we have by (9) that $\psi'(w) \equiv 0$. Therefore $\psi(w) \equiv$ constant, and by allowing w to be real in (11), we see that this constant must be pure imaginary:

$$\varphi_3(w) = \overline{\varphi_3(\bar{w})} + iC.$$

Hence

$$\Re\varphi_3(w) = \Re\overline{\varphi_3(\bar{w})} = \Re\varphi_3(\bar{w}). \tag{12}$$

The equations

$$x = \Re\varphi_1(w), \qquad y = \Re\varphi_2(w), \qquad z = \Re\varphi_3(w) \tag{13}$$

define the required minimal surface $\mathfrak{M}$ coinciding with M for $v > 0$. We see by (5) and (12) that the law of prolongation of M is

$$x(u, -v) = -x(u, v), \quad y(u, -v) = -y(u, v), \quad z(u, -v) = z(u, v) \tag{14}$$

which represents a symmetry in the line l, as stated in our theorem.

[1] "The Analytic Prolongation of a Minimal Surface over a Rectilinear Segment of Its Boundary," *Duke Math. Jour.*, **5**, 21–29 (March, 1939).

We may take this occasion to observe that in the statement, loc. cit., p. 24, lines 12–16, the difference quotient must be interpreted as a simultaneous difference quotient: $\{f(u + h) - f(u + k)\}/(h - k)$, whose limit must be taken as $h, k \to 0$ together. The proof given of the existence of z_u on ab is easily adjusted to this interpretation.

[2] The exact meaning of this is discussed in the paragraph following formula (3).

[3] See the reference in footnote 2.

[4] The slit is along the u-axis from b (inclusive) to $+\infty$ and from a (inclusive) to $-\infty$.

THE AUTOMATIC SYNTHESIS OF SPEECH

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The synthesis of speech has long appealed to the mind of man. There is a detailed report by Von Kempelen[1] of his experiments with mechanical models of speaking machines around 1780. Since then many famous names including Wheatstone and Helmholtz have been associated with some form of speech synthesis. The automatic synthesis[2] of speech described in the present paper has been made possible by the powerful tech-

niques associated with the developments in communication circuits and apparatus in recent years.

This automatic synthesis of speech is carried out in two steps, the remaking of the speech by an electrical synthesizing circuit being preceded by an electrical analysis of the speech it is desired to remake. The overall process thus uses electrical circuits to copy a man repeating speech as he hears it. By analogy the analyzer serves for an artificial ear and the synthesizer for an artificial vocal system. Actually the synthesizer was designed to be a somewhat simplified equivalent of the human vocal system in the essential steps of speech production whereas the analyzer was designed on the basis of working satisfactorily with the synthesizer rather than as an artificial equivalent of the ear. In view of this line of development the broad features of the synthesizer will be touched upon before giving a more detailed discussion of the analyzing method used.

The synthesizer fashions its synthetic speech out of two basic sound streams, namely, a buzzer-like tone from a relaxation oscillator which for brevity will hereafter be referred to as a buzz and a noise from random vibrations in the electrical current in a gas tube which will hereafter be referred to as a hiss from the nature of its sound. The speech sounds in general can be fairly well simulated with one or the other of these basic streams of sound as the raw material. The buzz consists of a fundamental frequency and its harmonics and is employed for making the voiced sounds, while the hiss has a random nature corresponding to the presence of components at all vibration rates in the audible range used here and is employed for the unvoiced sounds. In fashioning the synthetic speech sounds from these sound streams, two modulating or control processes are applied. The fundamental frequency of the buzz is varied to simulate the pitch of voiced speech with its inflection while the spectral variation of amplitude with frequency for the various speech sounds, whether voiced or unvoiced, is simulated by controlling the relative amounts of power transmitted in fixed frequency bands. With this general procedure for constructing synthetic speech as a background the more important details of the analyzing and synthesizing operation will be filled in.

The Speech Analyzer.—Figure 1 shows the overall circuit for remaking speech with the analyzer at the left and the synthesizer at the right. Electrical speech waves from the microphone are analyzed for pitch by the top channel and for spectrum by a group of channels at the bottom.

In the pitch analysis the fundamental frequency, which for simplicity will be called the pitch, is measured by a circuit containing a frequency discriminating network for obtaining the fundamental frequency in reasonably pure form, a frequency meter for counting by more or less uniform pulses the current reversals therein and a filter for eliminating the actual speech frequencies but retaining a slowly changing current

that is a direct measure of the pitch. Unvoiced sounds, whether in whispering or the unvoiced sounds of normal speech, have insufficient power to operate the frequency meter. The output current of the pitch channel is then a pitch-defining signal with its current approximately proportional to the pitch of the voiced sounds and equal to zero for the unvoiced sounds.

The analysis for spectrum is made easier by inserting the indicated predistorting equalizer having the loss characteristic given in figure 2 to obtain a fairly uniform amount of power in the various channels. While

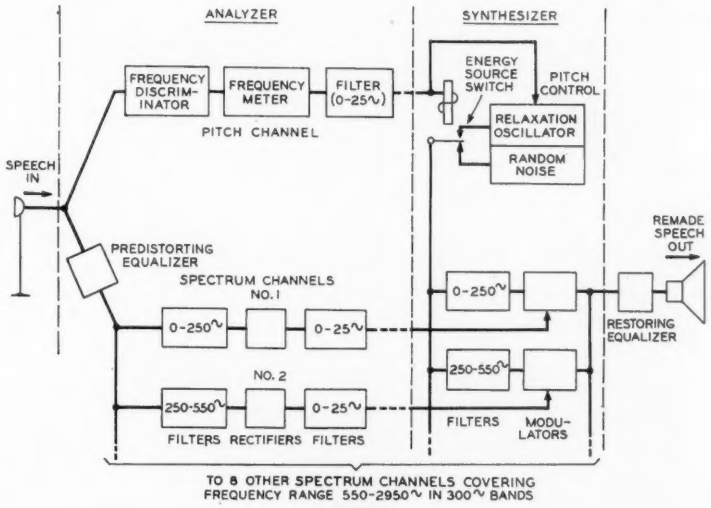

FIGURE 1

Schematic circuit for the automatic synthesis of speech.

this equalizer results in a better working of the spectrum circuits it produces no net distortion in the speech characteristic because its effect is annulled by the restoring equalizer at the end of the synthesizer. There are 10 spectrum-analyzing channels, the first one handling the frequency range 0–250 cycles and the other nine, the bands, 300 cycles wide, extending from 250 cycles to 2950 cycles, the top frequency being chosen as representative of commercial telephone circuits. Each spectrum-analyzing channel contains the proper band filter followed by a rectifier for measuring the power therein and a 25-cycle low-pass filter for retaining the current indicative of this power but eliminating any of the original speech frequencies.

The operation of the analyzer is illustrated in figure 3 by a group of oscillograms taken in analyzing the sentence "She saw Mary." To insure

that the same speech was analyzed in obtaining the various oscillograms, the sentence was recorded on a high quality magnetic tape recorder and reproductions therefrom used for the oscillograph tracings. The speech wave input to the analyzer after passing the predistorting equalizer is shown in the line next to the bottom while the output is shown in the other oscillogram traces, the pitch-defining signal being below the speech wave and the 10 spectrum-defining signals above it. For convenient reference the oscillograms are lined up together whereas in the actual circuit the speech-defining signals lag about 17 milliseconds behind the speech input wave. While the inaudible speech-defining output signals contain all the essential speech information of the input wave, it is noted that they are slow-changing, in this way corresponding to lip or

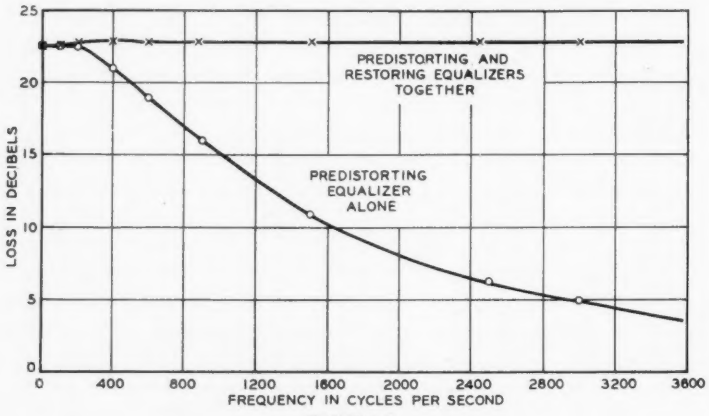

FIGURE 2

Loss characteristics of the equalizers in the circuit of figure 1.

tongue motions, as contrasted with the rapid-changing speech wave itself composed of the much higher audible vibration rates. The dropping of the pitch to zero for the unvoiced sounds "sh" and "s" is also readily seen.

The Speech Synthesizer.—Figure 3 shows the effect of the synthesizing process as well as of the analyzing process. In the analyzer the speech wave is the input and the 11 speech-defining signals are the output while in the synthesizer the 11 speech-defining signals are the input and the speech wave the output as well as can be indicated on such a condensed scale.

The steps in speech synthesis mentioned previously can be followed readily in the synthesizer circuit shown at the right of figure 1. The relaxation oscillator is the source of the buzz and the random noise circuit the source of the hiss. The hiss is connected in circuit for unvoiced sounds

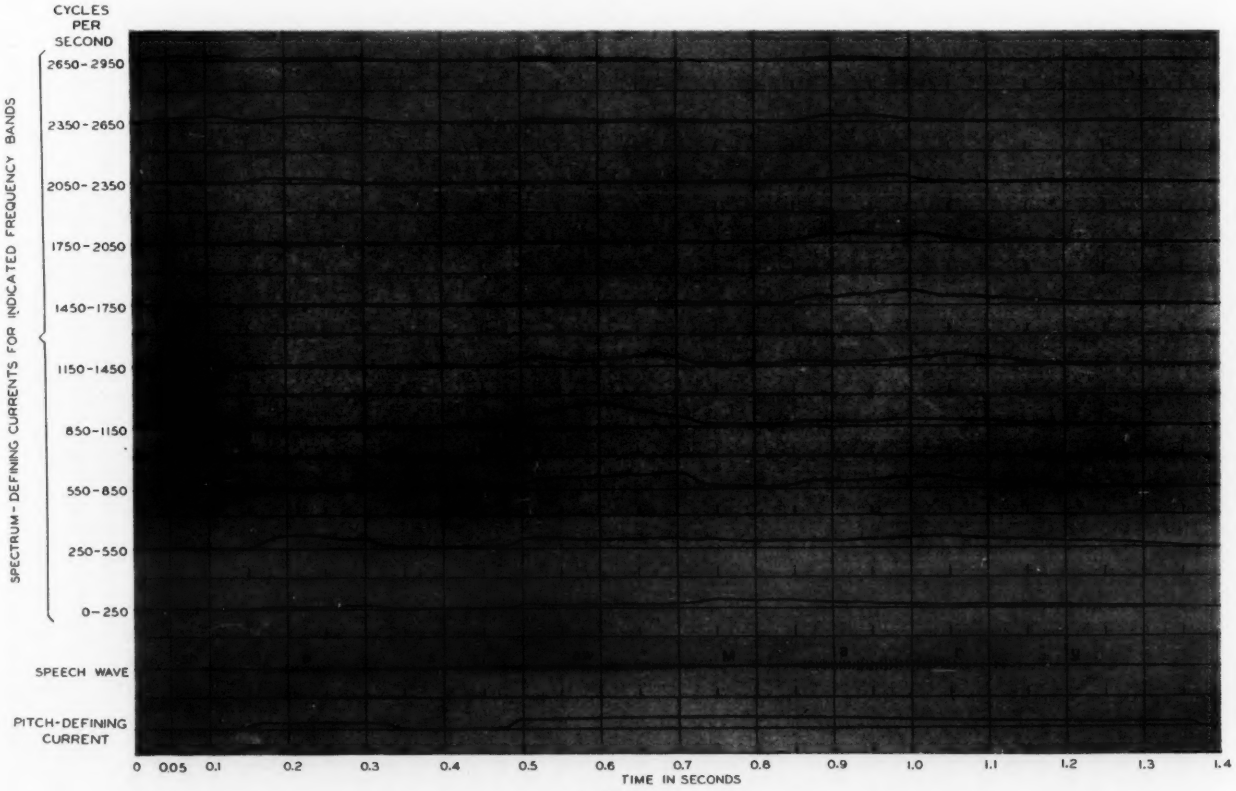

FIGURE 3

Oscillogram of the speech wave (0–3000 cycles) and the speech-defining signals (0–25 cycles) for the sentence "She saw Mary."

and for quiet intervals. (In the latter case the zero sound output from the synthesizer results from the zero currents in the spectrum channels.) When a voiced sound is analyzed a pitch current other than zero is received from the analyzer with the result that the buzz is set for the correct pitch by the "pitch control" on the relaxation oscillator and at the same time the relay marked "energy source switch" operates switching from the hiss source to the buzz source.

The outputs from the spectrum-analyzing channels are fed to the proper synthesizing spectrum controls with the band filters lined up to correspond. The power from the synthesizer energy sources in these various bands is then passed through modulators under the control of the spectrum-defining currents so that the power output in each filtered band from the synthesizer is proportional to that measured by the analyzer in the original speech. After the restoring equalizer there is, then, synthetic speech having approximately the same pitch and the same spectrum as the original speech. The synthetic speech lags the original speech by about 17 milliseconds due to the inherent delay in electrical circuits of the types used.

Application.[3]—It may be expected that this device will prove useful in making speech studies, especially those of a type in which the ear is the ultimate judge. With the speech-defining signals now made available as independent variables, they are put under the control of the experimenter. Thus speech can not only be reproduced but literally remade to new specifications. Observations may be made on the transformation of voiced speech to whispered speech, on the independent contributions of voiced and unvoiced sounds, and on the separate parts played by spectrum and inflection. The association of intelligibility with spectrum and emotional content with pitch change can be made strikingly apparent. The voice pitch may be greatly decreased or greatly increased; its range may be augmented, diminished or held to a perfect monotone; its inflections may be inverted. Radical speech changes are possible such as splitting the analyzing equipment to obtain hybrid voices formed with the pitch of one talker and the spectrum of another.

As to the engineering possibilities which may grow out of the application of the principles employed in this device, it is hard to predict at the present time. The speech-defining currents do have features of simplicity and inaudibility which may open the way to new types of privacy or to a reduction in the frequency range required for the transmission of intelligible telephonic speech.

[1] *Mechanismus der menschlichen Sprache nebst der Beschreibung seiner sprechenden Maschine*, 1791.

[2] The synthesizing apparatus in its earlier stages of development was demonstrated during an address by Dr. Frank B. Jewett at the Harvard Tercentenary Conference of

Arts and Sciences, September 11, 1936, on "The Social Implications of Scientific Research in Electrical Communication," *Bell Telephone Quarterly*, **15**, 205–218 (1936), and *Scientific Monthly*, **43**, 466–476 (1936). A description of this demonstration is given in a brief article on "Synthesizing Speech," by H. Dudley in the *Bell Laboratories Record*, **15**, 98–102 (1936).

[3] When this paper was presented at the 1939 Spring meeting, phonograph records were used to illustrate some of these possibilities as well as to show the important steps in the automatic synthesis of speech.

THE PHOTOGRAPHY OF INTERATOMIC DISTANCE VECTORS AND OF CRYSTAL PATTERNS

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1. *Introduction.*—In a recent note,[1] W. L. Bragg has described a method of optically accomplishing the two-dimensional Fourier summation

$$\rho_{x,y} = \Sigma\Sigma F_{h0l} \cos\left\{\frac{2\pi hx}{a} + \frac{2\pi lz}{c} + \alpha_{h0l}\right\} \qquad (1)$$

which gives the electron density, ρ, of all points, x, z, of a crystal structure, projected on (010). In Bragg's method, a complete zero-layer reciprocal lattice level is laid out on a thin brass plate, and at each lattice point, a hole is drilled having an area proportional to F_{h0l}. This perforated plate is placed between a pair of lenses and illuminated by a monochromatic point source placed at the focus of the first lens. The wavelets diffracted from the holes in the brass plate have the correct amplitudes and spacings to give diffraction equivalent to the above Fourier summation, so that the second lens gives an image of the electron density projected on (010), i.e., a picture of the crystal structure itself. The arrangement provides a practical method of rapidly synthesizing crystal structures from x-ray diffraction data in the cases of crystals, all of whose diffraction spectra have the same phase, i.e., $\alpha_{h0l} = 0$. This situation obtains in cases of crystals having a center of symmetry in the plane of the projected pattern, and having sufficiently heavy atoms at these symmetry centers.

In the present note, suggestions are made for (*a*) extension of the method to cases where α is either 0 or π, i.e., to any crystal having a projected center of symmetry, (*b*) extension of the method to Patterson and Harker summations for determining interatomic distance vectors and (*c*) reduction of many of the steps involved to purely photographic processes, so

that it can fairly be said that it is possible to take an actual photograph of the ends of interatomic distance vectors and of the crystal pattern itself.

2. *Control of Phase.*—The following device fulfills the requirement of developing a phase angle, α, of either 0 or π at will: The grating is placed between crossed polaroids (or other corresponding device). The vibration directions of the polarizer and analyzer are indicated by OP and OA, figure 1. A small strip of polaroid is also placed over each hole in the grating. For producing $\alpha_{h0l} = 0$, the strip is laid with its vibration direction at 45° (OS), and for producing $\alpha_{h0l} = \pi$, the strip is laid at 135° (OT), with the direction of the analyzer. Thus an original polarized vibration having amplitude OP is resolved to OS for holes which are to give rise to $\alpha_{h0l} = 0$, and resolved to OT for holes which are to give rise to $\alpha_{h0l} = \pi$. The analyzer only permits components OU and OV, respectively, to arrive at the focus of the second lens.

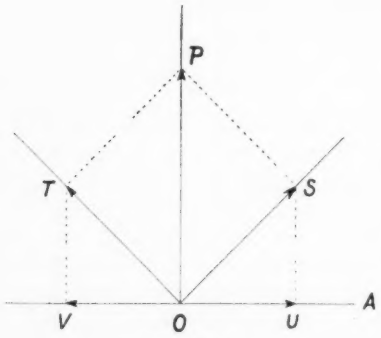

FIGURE 1

Thus with the aid of a set of polaroid strips equal in number to the holes in the grating, the phase of each diffracted wave can be set at either 0 or π, and this enables Bragg's device to be used with any crystal whose projected pattern involves a center of symmetry. The analysis of such a crystal is thus reduced to trying out all the possible phase combinations of 0 and π. If there are n symmetrically unequivalent reciprocal lattice points, then there are 2^n phase combinations, and all of these can be tried and the results examined or photographed in comparatively short order. The correct crystal structure lies among these. This number of combinations becomes greatly reduced as the projected crystal pattern becomes more symmetrical.

3. *Application to Two-Dimensional Patterson Synthesis.*—Patterson has shown[2,3] that the summation

$$A_{x,z} = \Sigma\Sigma \mid F_{h0l} \mid^2 \cos\left\{\frac{2\pi hx}{a} + \frac{2\pi lz}{c}\right\} \qquad (2)$$

is a function having maxima whose vector distances from the origin represent interatomic distance vectors in the crystal, projected on (010). This summation is identical with (1) except that the coefficient, F_{h0l}, is

replaced by its square. An optical picture of the ends of the interatomic vectors can thus be produced by making the areas of the holes in the brass plate of Bragg's method proportional to $|F_{h0l}|^2$, rather than to F_{h0l}.

4. *Application to Patterson-Harker Syntheses.*—Harker[4] has developed a type of three-dimensional Patterson synthesis of special use for determining the components of vectors between atoms related by space-group symmetry elements. In these syntheses, the summation of $|F_{hkl}|^2$ remains three-dimensional while the summation of the phase factor, $\cos 2\pi\left\{\frac{hx}{a}+\frac{ky}{b}+\frac{lz}{c}\right\}$, becomes two-dimensional (in some cases degenerating to one-dimensional) and of the form $\cos 2\pi\left\{\frac{hx}{a}+\frac{lz}{c}\right\}$. If the additional dimension of the $|F_{hkl}|^2$ summation is first carried out, then the Harker syntheses reduce to the form

$$A_{xz} = \Sigma\Sigma[\Sigma(\pm 1)^k |F_{hkl}|^2] \cos 2\pi\left\{\frac{hx}{a}+\frac{lz}{c}\right\}. \qquad (3)$$

This is also of form similar to (1) and can be carried out by the Bragg device by making the areas of the holes proportional to $\Sigma(\pm 1)^k |F_{hkl}|^2$. This term expresses the total real computation involved, and amounts to an algebraic addition. The optical diffraction grating accomplishes the rest of the Fourier summation.

5. *The Direct Photography of Appropriate Gratings.*—In actually performing any of the above summations, a large number of preliminary operations is involved. These include indexing spots on the x-ray photographs, measuring their positions and computing the reciprocal cell constants, measuring the total x-ray energy represented by each spot, transformation of these energies received into $|F_{hkl}|^2$'s, by computing and multiplying each by at least the Lorentz and polarization factors. In the case of producing a picture of the crystal pattern, each $|F_{hkl}|^2$ must finally be reduced to $|F_{hkl}|$. All these steps can be avoided, making it possible to photograph directly the ends of interatomic vectors as well as the crystal structures themselves.

deJong and Bouman[5, 6, 7, 8] have recently described a camera for taking direct photographs of the reciprocal lattice, level by level. These photographs are plane lattices whose points are blackened to an exposure value proportional to $|F_{hkl}|^2$ times (chiefly) the Lorentz and polarization factors. We can arrange to eliminate the appearance of these factors (and indeed introduce a temperature factor if desired) by introducing a very rapidly rotating sector between crystal and film, such that the reflections are suppressed during a time proportional to the enhancement by the Lorentz and polarization factors. The result is a series of plane lattices whose point densities correspond to the $|F_{hkl}|^2$'s, and whose background is nil if the

radiation has been properly monochromatized.[9] If this original film is called the negative, then a positive print (preferably a projection) made by the method of Dawton,[10] is an opaque film having transparent spots at reciprocal lattice points, whose transmissions are proportional to $|F_{hkl}|^2$.

If this film represents a zero level, then it is the direct photographic equivalent of Bragg's brass plate, except that the transmissions of the holes are proportional to $|F_{h0l}|^2$ instead of $|F_{h0l}|$. The focused diffraction picture from this film immediately gives a two-dimensional Patterson synthesis projected on that plane. If the transmissions, $|F_{h0l}|^2$, are transformed to $|F_{h0l}|$ and the signs of the phases controlled as indicated in section 2, then the diffraction picture is a *direct photograph of the crystal structure itself*.

The transformation from $|F_{hkl}|^2$ to $|F_{hkl}|$ can be accomplished in several possible ways:

(*a*) A second negative can be made by projection-printing from the first positive, one spot at a time, the light which goes through each spot being filtered through a photocell operated valve, whose characteristic is to reduce an intensity to its square root. A positive made from this negative gives the required $|F_{hkl}|$ transmission.

(*b*) Either negative or positive might be treated with an appropriate "intensifying" agent, whose function is to preferentially reduce the final transmission of the higher intensities. If a treatment of appropriate characteristics can be experimentally found, this plan would offer a simpler transformation from $|F_{hkl}|^2$ to $|F_{hkl}|$.

One of the characteristics of x-ray photographs is that they lack a lattice point at the origin. In the applications already mentioned, the missing point in question is 000. This contributes equally to all points on the crystal pattern picture, so that the only effect of its absence is to reduce the positive intensity of all points by the same amount. This lattice point can be approximately introduced into the film artificially by making a hole in the emulsion of the positive film. Since the transmission of 000 is the maximum possible transmission, it can be approximated by making it somewhat larger than the greatest recorded transmission. In Patterson syntheses, reduction of background enhances the maxima. This can be accomplished by reducing an artificial transmission of 000 by two polaroids having a variable angle between vibration directions.

6. *Photographic Plane Gratings for Patterson-Harker Syntheses.*—Plane gratings for any of the Patterson-Harker syntheses can be prepared by first making positives from appropriate deJong and Bouman negatives and then projection printing these in appropriate registry on a single negative. A new positive made from this is the required grating. The superposition effects the $\Sigma|F_{hkl}|^2$ summation provided the synthesis is made for a zero layer. (The glide-plane case, which Harker synthesizes on level $\frac{1}{2}$,

can also be made on the zero level provided the glide plane is perpendicular to the level and the glide component is parallel with the level. The desired maxima then have a glide-component coördinate of $\frac{1}{2}$ and the desired unknown coördinate is normal to this.) In the case of the two-fold screw axis, the intensity summation takes the form $\Sigma(-1)^k \mid F_{hkl} \mid^2$. This requires the algebraic summation of negative and positive intensities. This can be provided for in printing by offsetting the even and odd levels slightly with respect to one another, making, in effect, a doublet spot. Over the positive parts of the spots are placed polaroid strips with vibration direction at 45° and over negative parts, polaroids at 135° (with appropriate offset, the entire polaroid covering is provided by a few long strips). By placing the print plus polaroid strips between polaroids crossed at 0° and 90°, the required algebraic summation is effected with the trigonometric summation.

It should be observed that in n-levels, lattice points are missing in the region of the level origin. The error due to missing reflections directly above 000 is not important, as pointed out previously, but the circle of missing points enlarges slowly with level height and errors are introduced also due to these. Fortunately they become of lesser importance in the higher levels, because usually the intensities themselves become smaller. Use of shorter wave-lengths tends to alleviate this error. If an equi-inclination technique is used, it can be avoided completely. In any case, corrections may be provided by cutting out appropriate portions of other negatives and adding them to the appropriate lattice positions of the negatives to be printed.

7. *Alternative Procedures.*—The grating can also be prepared from sets of films made by other x-ray recording methods, for example the equi-inclination Weissenberg method. The camera is first equipped with a rapidly oscillating shutter for providing differential exposures to correct for the Lorentz and polarization factors. Positive prints then provide sets of transmissions $\mid F_{hkl} \mid^2$ for the summation, but they are not arranged in grating form. The algebraic summation and production of the grating may be carried out at the same time by placing the summable $\mid F_{hkl} \mid^2$'s so that they simultaneously affect photocells, whose outputs may be arranged to add algebraically. The net output may be permitted to actuate an optical diaphragm and produce blackening at a lattice point position of a new negative. The positive print of this negative is the required grating for any desired Patterson-Harker analysis.

[1] W. L. Bragg, "A New Type of 'X-Ray Microscope,'" *Nature*, **143**, 678 (1939).

[2] A. L. Patterson, "A Fourier Series Method for the Determination of the Components of Interatomic Distances in Crystals," *Phys. Rev.*, **46**, 372–376 (1934).

[3] A. L. Patterson, "A Direct Method for the Determination of the Components of Interatomic Distances in Crystals," *Zeit. Krist.* (A) **90**, 517–542 (1935).

[4] David Harker, "The Application of the Three-Dimensional Patterson Method and the Crystal Structures of Proustite, Ag_3AsS_3, and Pyrargyrite, Ag_3SbS_3," *Journ. Chem. Phys.*, **4**, 381–390 (1936).

[5] W. F. deJong, J. Bouman, and J. J. deLange, "X-Ray Photography of Zero-Order Reciprocal Net Planes of a Crystal," *Physika*, **5**, 188–192 (1938).

[6] W. F. deJong and J. Bouman, "Das Photographieren von reziproken Kristallnetzen mittels Röntgenstrahlen," *Zeit. Krist.* (A) **98**, 456–459 (1938).

[7] W. F. deJong and J. Bouman, "Das Photographieren von reziproken Netzebenen eines Kristalles mittels Röntgenstrahlen," *Physika*, **5**, 220–224 (1938).

[8] J. Bouman and W. F. deJong, "Die Intensitäten der Punkte einer photographierten reziproken Netzebene," *Physika*, **5**, 817–832 (1938).

[9] I. Fankuchen, "A Condensing Monochromator for X-Rays," *Nature*, **139**, 193 (1937).

[10] Ralph H. V. M. Dawton, "The Integration of Large Numbers of X-Ray Crystal Reflections," *Proc. Phys. Soc.*, **50**, 919–925 (1938).

THE AERODYNAMICS OF REACTING SUBSTANCES

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Speaking broadly, the subject of aerodynamics is concerned not only with the forces exerted by a single fluid on solid bodies but also with the behavior of mixtures such as those which occur in the gasoline engine and in the atmosphere. In a complete study of the motion of a fluid attention must be paid to reactions between its constituents, evaporation, conduction and radiation of heat, diffusion, viscosity and other phenomena.

It is uncertain to what extent equations governing the various processes can be derived from a single variational principle as some of the phenomena mentioned are known to present difficulties, but the situation may be clarified a little by an examination of the equations derived from a variational principle of a very general type.

We consider n superposed substances $S_1, S_2, \ldots S_n$. The density of the substance S_r at the point (x, y, z) at time t will be denoted by ρ_r and a set of component velocities in an irrotational motion of this substance will be supposed to be derivable from a potential, ϕ_r, which is a function of x, y, z and t. The changes taking place in the complex will be regarded as governed more or less by a governing function, f, which is a function of x, y, z, t and of $\rho_1, \rho_2, \ldots \rho_n, \phi_1, \phi_2, \ldots \phi_n$. Writing $u_r = \dfrac{\partial \phi_r}{\partial x}$, $v_r = \dfrac{\partial \phi_r}{\partial y}$, $w_r = \dfrac{\partial \phi_r}{\partial z}$ we consider the variational principle

$$O = \delta \int d(x, y, z, t)\left[f - \sum_{s=1}^{n} \rho_s\left(\frac{\partial \phi_s}{\partial t} + {}^1/_2 u_s{}^2 + {}^1/_2 v_s{}^2 + {}^1/_2 w_s{}^2\right)\right] = \delta \int d(x, y, z, t) L. \quad (1)$$

in which the quantities $\rho_1, \rho_2, \ldots, \rho_n, \phi_1, \phi_2, \ldots, \phi_n$ are to be varied independently.

The equations of Euler and Lagrange for this variational problem are

$$o = f^s - \left(\frac{\partial \phi_s}{\partial t} + {}^1/_2 u_s{}^2 + {}^1/_2 v_s{}^2 + {}^1/_2 w_s{}^2\right), \; s = 1, 2, \ldots, n \quad (2)$$

$$o = f_s + \frac{\partial \rho_s}{\partial t} + \frac{\partial}{\partial x}(\rho_s u_s) + \frac{\partial}{\partial y}(\rho_s v_s) + \frac{\partial}{\partial z}(\rho_s w_s), \; s = 1, 2, \ldots, n \quad (3)$$

where the symbol f^s is used to denote $\partial f/\partial \rho_s$ and the symbol f_s is used to denote $\partial f/\partial \phi_s$.

Differentiating the first equation with respect to x we get

$$o = df^s/dx - (\partial u_s/\partial t + u_s \partial u_s/\partial x + v_s \partial u_s/\partial y + w_s \partial u_s/\partial z) \quad (4)$$

where $df^s/dx = \partial f^s/\partial x + \sum_{r=1}^{n} (f^{rs} \partial \rho_r/\partial x + f_r^s \partial \phi_r/\partial x)$.

Let us now write

$$p = f - \sum_{r=1}^{n} \rho_r \partial f/\partial \rho_r, \quad (5)$$

then

$$dp/dx = \partial f/\partial x - \sum_{r=1}^{n} \rho_r \partial f^r/\partial x + \sum f_r \partial \phi_r/\partial x - \sum \rho_r (f^{rs} \partial \rho_s/\partial x + f_s^r \partial \phi_s/\partial x)$$

$$= \partial f/\partial x - \sum_{r=1}^{n}\left[\frac{\partial}{\partial t}(\rho_r u_r) + (\partial/\partial x)(\rho_r u_r{}^2) + (\partial/\partial y)(\rho_r u_r v_r) + (\partial/\partial z)(\rho_r u_r w_r)\right]. \quad (6)$$

This is one of the dynamical equations for the system as a whole. The quantity p can be regarded as the total pressure. The mean motion (u, v, w) representing the motion of the center of mass of each fluid element is generally rotational until the differences in velocity of the constituents S_r are annulled.

Adding the equations (3) we see that Lavoisier's principle of the indestructibility of mass is fulfilled in the complete system if

$$\sum_{s=1}^{n} f_s = 0. \quad (7)$$

This equation is satisfied if f is a function of the differences $\phi_1 - \phi_s$. In the simple case in which $f = \sum_{s=1}^{n} F_s(\rho_s, \phi_1, \phi_2, \ldots, \phi_n)$ we may write

$$p_s = F_s - \rho_s \partial F_s / \partial \rho_s$$
$$p = \sum_{s=1}^{n} p_s. \tag{8}$$

The quantity p_r can then be regarded as the partial pressure of the constituent S_r. We have also

$$(\partial/\partial t)(\rho_r u_r) + (\partial/\partial x)(\rho_r u_r^2) + (\partial/\partial y)(\rho_r u_r v_r) + (\partial/\partial z)(\rho_r u_r w_r) = \partial F_r/\partial x - \partial p_r/\partial x. \tag{9}$$

These equations are like the ordinary dynamical equations for a single gas.

The principle is readily extended to the case of constituents in rotational motion.[1] The quantity under square brackets in (1) is replaced by

$$f - \sum_{s=1}^{n} \rho_s (D_s \phi_s + \alpha_s D_s \beta_s - {}^1/_2 u_s^2 - {}^1/_2 v_s^2 - {}^1/_2 w_s^2) \tag{10}$$

where $D_s = \partial/\partial t + u_s \partial/\partial x + v_s \partial/\partial y + w_s \partial/\partial z$,

and f is now a function of $x, y, z, t, \rho_1, \rho_2, \ldots, \rho_n, \phi_1, \phi_2, \ldots, \phi_n, \alpha_1, \alpha_2, \ldots, \alpha_n, \beta_1, \beta_2, \ldots, \beta_n, u_s, v_s, w_s \ldots$. In forming the equations of Euler and Lagrange the quantities $\rho_s, \phi_s, \alpha_s, \beta_s, u_s, v_s, w_s$ are to be varied independently. The principle may also be extended to the case in which the axes are those at a place on the rotating earth. The form of the Lagrangian function is readily inferred from the remark in the author's review of Ertel's book.

It is thought that these variational principles may be of some use in obtaining the characteristics of the set of partial differential equations characterizing the motion and, in particular, for the determination of the velocity and properties of a surface of discontinuity in the atmosphere or in an explosion engine. It will be seen from equations (3) that when the quantities f_s are not zero the equation of continuity for a constituent substance is not satisfied. This allows, then, for the production or destruction of the substance by a chemical transformation and the generalized equation of continuity should also be a generalized form of the law of mass action of chemical dynamics. Such an equation should be applicable in particular to the reaction zone which forms a region of continuity between burnt and unburnt gases in an explosion. It is just in such a region that a more complete treatment than that usually given is desirable.

In their recent work Lewis and von Elbe have devoted some attention to the part played by diffusion. To include diffusion in the present analy-

sis new terms must be added to the Lagrangian function L. If we add terms of type

$$\sum_{s=1}^{n} G_s\left(\frac{\partial \rho_s}{\partial x}\frac{\partial \phi_s}{\partial x} + \frac{\partial \rho_s}{\partial y}\frac{\partial \phi_s}{\partial y} + \frac{\partial \rho_s}{\partial z}\frac{\partial \phi_s}{\partial z}\right)$$

the left-hand side of equation (3) must be replaced by

$$\frac{\partial}{\partial x}\left(G_s\frac{\partial \rho_s}{\partial x}\right) + \frac{\partial}{\partial y}\left(G_s\frac{\partial \rho_s}{\partial y}\right) + \frac{\partial}{\partial z}\left(G_s\frac{\partial \rho_s}{\partial z}\right)$$

and a term

$$-\left[\frac{\partial}{\partial x}(G_s u_s) + \frac{\partial}{\partial y}(G_s v_s) + \frac{\partial}{\partial z}(G_s w_s)\right]$$

must be added to the right-hand side of equation (2). Thus in a non-uniform flow there may be forces on a constituent which arise from diffusion.

[1] An appropriate form of the principle was suggested by the author in a physical seminar at the California Institute of Technology in 1938 and also in a review of H. Ertel's "Methoden und Probleme der Dynamischen Meteorologie," *Zentralblatt für Mathematik und ihre Grenzgebiete*, **18**, 311 (1938). The analysis for the case of a single gas has been given in detail by H. Ertel, *Meteorologische Zeit.*, 105–108 (1939).

[2] Bernard Lewis and Guenther von Elbe, "Combustion, Flames and Explosion of Gases," Cambridge Univ. Press (1938). See also J. B. Zeldovich and D. A. Frank-Kameneckij, "On the Theory of Uniform Flame Propagation," *Compt. rend. (Doklady) de l'acad. des sciences de l'U. R. S. S.*, **19**, 693–697 (1938).

A QUESTION IN GENERAL RELATIVITY

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I.—The following question, which provides an illuminating application of general relativity to electrodynamics, has been put to me by Professor Oppenheimer. Consider two concentric spheres with equal and opposite total charges uniformly distributed over their surfaces. When the spheres are at rest, the electric and magnetic fields outside the spheres vanish. When the spheres are in uniform rotation about an axis through their center, the electric field outside vanishes, while the magnetic field does not, since the magnetic moment of each of the spheres is proportional to the square of its radius. Suppose that the spheres are stationary; then an observer traveling in a circular orbit around the spheres should find no field, for since all of the components of the electromagnetic field tensor vanish in one coördinate system, they must vanish in all coördinate systems.

On the other hand, the spheres are rotating with respect to this observer, and so he should experience a magnetic field.[1]

Before discussing the resolution of this apparent paradox, we shall present some general remarks on the relativity of rotational motion.

II.—It is clear in the above arrangement that an observer A at rest with respect to the spheres does not obtain the same results from physical experiments as an observer B who is rotating about the spheres. The very fundamental difference between coördinate systems in which A and in which B are at rest is ascribed, in the general theory of relativity, to the effect of distant masses (extragalactic nebulae), which are at rest with respect to A, but in violent motion with respect to B. That this is a plausible explanation was shown by Thirring's[2] calculation of the motion of a free particle inside a thin spherical shell of matter in uniform rotation about an axis through its center. He found that forces very similar to centrifugal and Coriolis forces appeared, but that they were too small to represent the forces acting on a particle rotating with the same angular velocity as the shell, by a factor of the order of the ratio of the gravitational radius to the actual radius of the shell.

From this relatively simple calculation, one infers that if the gravitational field equations could be solved in a coördinate system in which the actual distribution of matter in the universe is in uniform rotation, one would obtain exactly the usual centrifugal and Coriolis accelerations for a particle. The far simpler procedure that is usually followed is to take the approximately Galilean metric (neglecting local masses) that we know gives the experimentally correct equations of motion of a particle in the coördinate system in which the distant masses are at rest, transform it to the rotating coördinate system, and use this transformed expression for the metric to compute the motion of a particle. Because of the covariance of the gravitational field equations, one feels sure that the two calculations would give identical results. Thirring's direct calculation, however, has the advantage of providing some insight into the effect of rotating distant masses in warping the expression for the metric from its Galilean form.

Similarly, we know experimentally that the fields outside the charged spheres vanish in system A (in which the spheres and the distant masses are at rest), and so the covariance of Maxwell's equations guarantees that the fields will also vanish outside the spheres in system B. It is of interest, however, to see by direct calculation how it is that the spheres, which are rotating with respect to system B, do not give rise to a magnetic field outside. To see this, we must of course know the expression for the metric in system B, and we shall obtain this by transformation from the (approximately) Galilean metric of system A. The warping of the expression for the metric thus obtained for system B can be ascribed to the rotation of the distant masses in this system.

III.—The covariant formulation of Maxwell's equations[3] is:

$$\frac{\partial F_{\mu\nu}}{\partial x^{\sigma}} + \frac{\partial F_{\nu\sigma}}{\partial x^{\mu}} + \frac{\partial F_{\sigma\mu}}{\partial x^{\nu}} = 0, \tag{1}$$

$$\frac{\partial}{\partial x^{\nu}} (\sqrt{-g}\, F^{\mu\nu}) = \sqrt{-g}\, J^{\mu}. \tag{2}$$

The coördinate transformation from system A (subscript 0) to system B rotating with angular velocity ω can be taken as (velocity of light set equal to unity):

$$\begin{aligned} x &= x_0 \cos \omega t_0 + y_0 \sin \omega t_0, \\ y &= -x_0 \sin \omega t_0 + y_0 \cos \omega t_0, \\ z &= z_0, \\ t &= t_0, \\ r^2 &= x^2 + y^2 + z^2 = x_0^2 + y_0^2 + z_0^2, \end{aligned} \tag{3}$$

so that if the metrical tensor has its Galilean form in system A, it assumes in system B the form:[4]

$$\begin{aligned} g_{11} = g_{22} = g_{33} = -1, \quad & g_{44} = 1 - \omega^2(x^2 + y^2), \\ g_{14} = g_{41} = \omega y, \quad & g_{24} = g_{42} = -\omega x, \end{aligned} \tag{4}$$

and all other $g_{\mu\nu}$ vanish. Since the determinant $g = -1$, the electromagnetic field equations (1) and (2) are unaltered by the transformation (3). But the connections between the covariant and contravariant components of the field tensor depend on the metrical tensor:

$$F^{\mu\nu} = g^{\mu\alpha} g^{\nu\beta} F_{\alpha\beta}, \tag{5}$$

and so the field equations are different in the two coördinate systems when written entirely in terms of either the covariant or the contravariant components.

The calculations can be made in terms of any one set of components: covariant, contravariant or mixed.[5] We shall use here the covariant form of the tensor since the field equations (1) then have their usual structure, and the extra terms in equations (2) can be put in the form of an additional current. If we define:

$$\begin{aligned} F_{23} = H_x, \quad F_{31} = H_y, \quad F_{12} = H_z, \\ F_{14} = E_x, \quad F_{24} = E_y, \quad F_{34} = E_z, \end{aligned} \tag{6}$$

we find from (4) and (5) that:

$$\begin{aligned}
F^{23} &= (1 - \omega^2x^2)H_x - \omega^2xyH_y - \omega xE_z, \\
F^{31} &= (1 - \omega^2y^2)H_y - \omega^2xyH_x - \omega yE_z, \\
F^{12} &= (1 - \omega^2x^2 - \omega^2y^2)H_z + \omega xE_x + \omega yE_y, \\
F^{41} &= E_x - \omega xH_z, \\
F^{42} &= E_y - \omega yH_z, \\
F^{43} &= E_z + \omega xH_x + \omega yH_y.
\end{aligned} \tag{7}$$

Then equations (1) have their usual form:[6]

$$\text{div } \mathbf{H} = 0, \quad \textbf{curl } \mathbf{E} + \frac{\partial \mathbf{H}}{\partial t} = 0, \tag{8}$$

while equations (2) may be written:

$$\text{div } \mathbf{E} - \rho = \sigma, \quad \textbf{curl } \mathbf{H} - \frac{\partial \mathbf{E}}{\partial t} - \mathbf{J} = \mathbf{j}, \tag{9}$$

where we define:

$$\begin{aligned}
\sigma &= 2\omega H_z + \omega x\frac{\partial H_z}{\partial x} + \omega y\frac{\partial H_z}{\partial y} - \omega x\frac{\partial H_x}{\partial z} - \omega y\frac{\partial H_y}{\partial z}, \\
j_x &= 2\omega^2yH_z - \omega E_y - \omega^2xy\frac{\partial H_x}{\partial z} - \omega^2y^2\frac{\partial H_y}{\partial z} - \omega x\frac{\partial H_z}{\partial t} + \\
&\qquad \omega^2(x^2 + y^2)\frac{\partial H_z}{\partial y} - \omega x\frac{\partial E_x}{\partial y} - \omega y\frac{\partial E_y}{\partial y} - \omega y\frac{\partial E_z}{\partial z}, \\
j_y &= -2\omega^2xH_z + \omega E_x + \omega^2x^2\frac{\partial H_x}{\partial z} + \omega^2xy\frac{\partial H_y}{\partial z} - \omega y\frac{\partial H_z}{\partial t} - \\
&\qquad \omega^2(x^2 + y^2)\frac{\partial H_z}{\partial x} + \omega x\frac{\partial E_x}{\partial x} + \omega y\frac{\partial E_y}{\partial x} + \omega x\frac{\partial E_z}{\partial z}, \\
j_z &= \omega^2yH_x - \omega^2xH_y + \omega^2y^2\frac{\partial H_y}{\partial x} - \omega^2x^2\frac{\partial H_x}{\partial y} - \omega y\frac{\partial H_z}{\partial t} + \\
&\qquad \omega^2xy\frac{\partial H_x}{\partial x} - \omega^2xy\frac{\partial H_y}{\partial y} + \omega y\frac{\partial E_z}{\partial x} - \omega x\frac{\partial E_z}{\partial y}.
\end{aligned} \tag{10}$$

For any spherically symmetrical distribution of charge that is stationary in system A (in particular for our two charged spheres), we have in system B:

$$J_x = \omega y\rho, \quad J_y = -\omega x\rho, \quad J_z = 0. \tag{11}$$

Substituting (10) and (11) into (9), one can either guess a solution or, more satisfyingly, obtain one directly by treating terms in ω as a perturbation, in which case it is possible to carry the perturbation calculation to arbi-

trarily high orders. The unperturbed solution ($\omega = 0$) that satisfies the boundary conditions (no singularities and vanishing at infinity) is:

$$\mathbf{H} = 0, \quad \mathbf{E} = \frac{4\pi \mathbf{r}}{r^3} \int_0^r \rho(\zeta)\zeta^2 d\zeta. \tag{12}$$

To obtain the fields to first order in ω, we notice that $\mathbf{H}$ is at most of order $\omega\mathbf{E}$, so that to this order we may write equations (10):

$$\begin{aligned} \sigma &= 0 \\ j_x &= -\omega E_y - \omega x \frac{\partial E_x}{\partial y} + \omega y \frac{\partial E_x}{\partial x} - \omega y \operatorname{div} \mathbf{E}, \\ j_y &= \omega E_x - \omega x \frac{\partial E_y}{\partial y} + \omega y \frac{\partial E_y}{\partial x} + \omega x \operatorname{div} \mathbf{E}, \\ j_z &= \omega y \frac{\partial E_z}{\partial x} - \omega x \frac{\partial E_z}{\partial y}. \end{aligned} \tag{13}$$

Putting the unperturbed fields (12) into (13), we obtain:

$$\begin{aligned} j_x &= -\omega y \operatorname{div} \mathbf{E} = -\omega y \rho = -J_x, \\ j_y &= \omega x \operatorname{div} \mathbf{E} = \omega x \rho = -J_y, \\ j_z &= 0, \end{aligned} \tag{14}$$

and equations (9) are satisfied by the fields (12) to the first order. This result is readily extended to arbitrarily high orders in ω, and it is seen that the fields (12) are an exact solution of the field equations (9) and (10) with the current (11). Since for our two oppositely charged spheres, the integral in (12) vanishes for values of r greater than the radius of the larger sphere, both the electric and magnetic fields vanish outside the spheres in system B, in agreement with the result obtained by transformation from system A.

It is of interest to note that the vanishing of the fields in this calculation is due to the cancellation of the actual current $\mathbf{J}$ with other terms (right side of (9)) that behave in this respect like a current. The appearance of this extra current (10) is due to the action of the rotating distant masses, via the metric, on the electromagnetic fields.

[1] From these remarks it is evident, for example, that one cannot calculate the magnetic field about a single charged rotating sphere by transforming the electrostatic field of such a sphere at rest.

[2] Thirring, *Phys. Zeits.*, **19**, 33 (1918); **22**, 29 (1921).

[3] Tolman, *Relativity, Thermodynamics and Cosmology* (Oxford, 1934), p. 259.

[4] The indices 1, 2, 3, 4 refer to x, y, z, t, respectively.

[5] It is the mixed tensor that gives the acceleration of a charged particle (cf. Tolman, reference 2, p. 260); however, any one form is readily obtained from any other.

[6] Bold-face symbols indicate ordinary three-dimensional vectors.

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